

A scalable method for multiplex LED-controlled synthesis of DNA in capillaries

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ABSTRACT

As research in synthetic biology and genomic sciences becomes more widespread, the need for diverse oligonucleotide populations has increased. To limit reagent cost, it would be advantageous to obtain high quality populations in minute amounts. Towards that end, synthesis of DNA strands in capillaries utilizing photolabile 3-nitrophenylpropyloxycarbonyl (NPPOC) chemistry and ultraviolet-light emitting diodes (UV-LEDs) was examined. Multiple oligonucleotides were made in single capillaries and were characterized by hybridization, sequencing and gene synthesis. DNA synthesized in capillaries was capable of being hybridized and signal intensities correlated with microarray data. Sequencing demonstrated that the oligonucleotides were of high quality (up to 44% perfect sequences). Oligonucleotides were combined and used successfully for gene synthesis. This system offers a novel, scalable method to synthesize high quality oligonucleotides for biological applications.

INTRODUCTION

The rapid growth of genomic sciences, and in particular synthetic biology, has heightened the need for synthetic DNA. Currently the cost of per-base synthesis of DNA constructs remains high and is a barrier to many large-scale studies. For many experiments, a flexible design with rapid turn-around is very important as well as the cost and the quality of the synthesized oligos. Today, the *de-novo* synthesis of long DNA constructs is based on the assembly of shorter construction oligomers typically 40-80 nt in length. Several companies offer oligos (and genes) and many central facilities have synthesis capabilities, often based on ABI (Applied Biosystems) or Mermade (BioAutomation) DNA synthesizers.

Standard DNA synthesis is based on acid labile phosphoramidite chemistry (1), and entails the use of a separate solid support (in the form of glass beads) for each oligonucleotide synthesized. Often this synthesis approach creates products in much larger amounts than needed and which require additional cleaning before use. In addition, the development of large-scale combinatorial studies requires the synthesis of large libraries of many different oligo sequences in small amounts. Traditional synthesis tools are not well suited for this kind of work, and new avenues leading to parallel synthesis are being actively investigated. DNA microarray chips synthesized through various methods (2-4) have been used as sources of oligos for DNA assembly (5,6). However, these chips are both relatively expensive and not readily available. Thus, a technique that could be easily set up in a laboratory to rapidly yield a number of low cost, high quality oligomers would provide an interesting avenue to many researchers. Point-of-use synthesis of DNA can complement large scale processes in a very effective way.

We have constructed short gene fragments by assembling oligomers eluted from photo-synthesized DNA microarray chips using a maskless technique (5). This approach yields hundred of thousands of different oligo sequences but requires specialized ultraviolet (UV) optics and processing tools. During the development of this technology, we examined the feasibility of a smaller-scale synthesis system better suited for studies not requiring massive libraries of oligomers. The use of light deprotection enables the synthesis of several different oligos in a single reaction chamber, and is thus ideally suited to parallel synthesis. However, there is a challenge in achieving a spatially controlled light distribution without resorting to complex optics and illumination sources. The combination of a capillary synthesis chamber with a set of discrete and independently controlled light sources—light-emitting diodes (LEDs)—provides the answer for a simple point-of-use synthesizer.

Capillaries have long been used in many biological applications, from high performance liquid chromatography (HPLC) to synthesis. Glass capillaries are available in a wide variety

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The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors

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of forms and sizes, can be easily functionalized, are inexpensive and provide a good surface/volume ratio. Such attributes have already been used in the field of DNA sequencing for genome-wide sequencing (7,8). The use of capillary gel electrophoresis for DNA sequencing has allowed for automation, greater throughput, and a reduction in reagent use. Other applications have also exploited capillaries in order to limit reagent cost and integrate technologies, such as PCR (9,10) and mass spectrometry (11). In addition, capillaries can be used either 'bare', i.e. without any loading, or filled with beads, either solid or porous. The use of beads has the advantage of greatly increasing the synthesis surface area.

LEDs have found a phenomenal growth in applications since their introduction in the '70s, from communication to illumination systems. In the last decade there has been an explosion in the use of LED excitation in compact fluorometric instruments for biological applications [capillary electrophoresis (12–14) and flow cytometry (15)]. Today, LEDs are available with spectra ranging from the infrared to the UV. UV-LEDs are particularly important for the application described here, as it is based on light-directed 3-nitrophenylpropyloxycarbonyl (NPPOC) chemistry (16), until now used in conjunction with digital mirroring systems (5,17–19). We believe that LEDs are a reliable alternative to costly UV lasers and/or lamps.

With this in mind, a capillary synthesis cell (CSC) was engineered to utilize capillaries for DNA synthesis in conjunction with NPPOC chemistry and UV-LEDs. The concept of the system is illustrated in Figure 1. The CSC was used to synthesize several DNA oligomers. The oligos were characterized by hybridization to target sequences, sequenced after amplification and used for assembly in longer constructs. These results show clearly that the technique is capable of producing DNA oligomers of quantity and quality suitable for many genomic applications. As discussed below, this technology opens and expands new avenues for rapid, point-of-use synthesis of DNA.

MATERIALS AND METHODS

The overall system concept is described in Figure 1. Figure 2 shows the implementation of the concept. For proof-of-principle, we developed a cell with three LED sources. The details of the synthesis process have been presented before, (5), the components discussed in detail here are optics and capillary integration.

LED illumination

LED sources are based on a small chip (typically 200–500 μm^2) of a direct-gap semiconductor material, in this case gallium nitride (GaN). When electrical current is injected through the junction, the recombination of the carriers generates light of energy essentially equal to the energy gap of the semiconductor. In the case of GaN, this corresponds to a wavelength of 365 nm, equivalent to the so-called mercury I-line. The main advantages of solid-state sources is in their spectral purity, high brightness, ruggedness, long lifetimes and high efficiency. There is little wasted radiation (mercury lamps radiate mostly in the infrared and visible), so that heat dissipation is considerably reduced. In addition, their compact size makes integration much simpler. The LEDs used in the current application are UV-LEDs (Roithner,

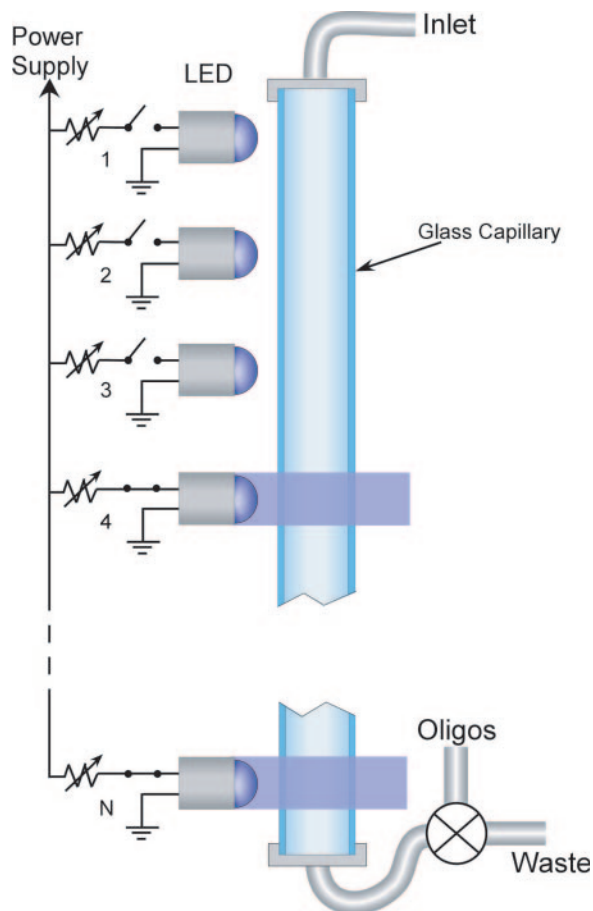


Figure 1. LED-based capillary Synthesis: a capillary is illuminated by a series of LEDs, independently addressed under the control of a computer. The UV light from each LED deprotects (activates) the region of the capillary immediately facing it. In this way, a single capillary becomes a multiplex synthesis system.

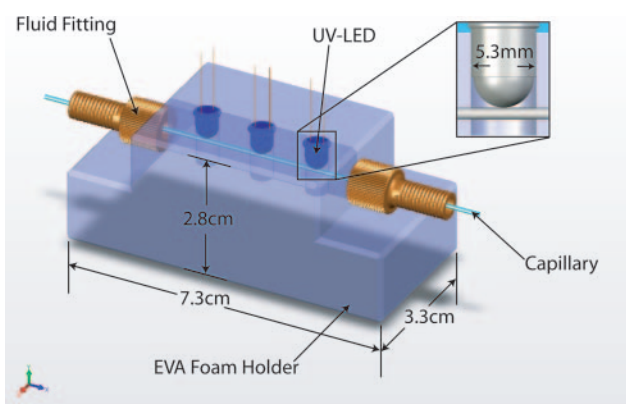


Figure 2. CSC: The CSC consists of an EVA foam casing (shown in blue) which houses the borosilicate glass capillary and supports the multiple LED units. The capillary is connected to a fluidics delivery system (Expedite DNA synthesizer; Applied Biosystems) by means of fluid fittings (shown in gold) LEDs were positioned to abut the surface of the capillary such that light was directed normal to the capillary axis (See expanded view).

UVLED365-10); the GaN chips are encased in plastic and generate a beam of 365 nm radiation with an opening of 10° .

An important aspect of LED illumination is the spectral purity. The spectrum has a half-width of 10 nm, and contains

no emission below 360 nm; this is very important to avoid damage to the DNA caused by shorter wavelength radiation. In the case of Hg I-line lamps, the deep-UV radiation must be filtered out. The LED used in this experiment generates 1.4 mW of radiation for a dissipated power of 72 mW; from an electrical point of view, the luminous output of the LED can be easily controlled by adjusting the current. Low-voltage operation (3.6 V supply at 20 mA) facilitates integration of control systems.

The cell shown in Figure 2 is made of structural foam. The LEDs are held in place by the foam matrix at a separation of 1 cm, and are directly contacting the capillary. The foam forms a cavity that confines the light around the exposure region. This simple and elegant design has several advantages, as discussed below.

As our method utilizes a light-directed chemistry, careful examination was performed to ascertain the presence and potential effects of scattered light. As shown in Figure 2, there are no optics between the LEDs and the capillary. Scattered light can be a major source of error in light directed synthesis since application of light at the wrong step would remove the photoprotecting groups and thereby allow for an extraneous coupling (causing an insertion error) to occur. Conversely, the lack of light at the required synthesis location will lead to the formation of single-point deletion errors. In the optical configuration used here, there are several details that need to be addressed. From Figure 1 we note that the light from the LED is incident perpendicular to the capillary. During exposure the capillary is filled with solvent (Nimble-Gen) which is refraction index-matched to the glass. Thus, there are no reflections at the inner surfaces of the capillary. The light that impinges upon the capillary is partly transmitted and partly reflected. The foam matrix acts as an optical trap that stops the light reflected and transmitted by the capillary from propagating to other adjacent sites. In addition, the angle of incidence on the capillary precludes any light-guide coupling modes because it is always larger than the critical angle. Thus the light is very effectively confined to the exposure region defined by the foam matrix and by the LED aperture cone. Scratches on the glass surface could act as scattering centers and launch light in the wave-guide modes, and therefore should be avoided. In support of this analysis, the distribution of sequence errors indicates that insertions were not predominant and in some cases were <5% of the total errors (data not shown).

CSC

The choice of the diameter of the capillary for use in the synthesis cell is dictated by the balance between two antagonistic

factors; the need to pump fluids with relatively low resistance and the need to minimize reaction volume. We selected a borosilicate glass capillary (Idaho Technology: outer diameter = 1.0 mm, inner diameter = 0.8 mm and length = 10.8 cm) with a volume of 55 μ l, (which is comparable to our MAS-based reaction cell). Our synthesis protocol is based on glass surfaces functionalized with monohydroxysilane. To produce monohydroxysilane capillary substrates, capillaries were gently shaken in a solution of 2% N-(3-triethoxysilylpropyl)-4-hydroxybutyramide for 4 h at RT, rinsed in 95% ethanol for 10 min, and cured in a vacuum oven at 120°C for 1 h. Capillaries were then fitted on either end with fluid fittings (Upchurch Scientific: 1/4-28 thread, PEEK, for 1/16" OD tubing, Super Flangeless fluid fitting over 1 mm sleeves). The capillary assembly was then inserted into a custom holder (Figure 2) to form the CSC. Construction of the CSC and its integration with the LED illumination system and the fluid delivery system was a multi-step process. For ease of capillary insertion and removal, the cell made of ethylene vinyl acetate (EVA) foam was engineered to allow for a length-wise addition of the tube. The fluid lines from an expedite DNA synthesizer (Applied Biosystems) were attached to the capillary via fluid fittings (Figure 2). The UV-LEDs were then inserted into the capillary holder until they abutted the outer surface of the capillary (Figure 2). We note that this method can be easily extended to longer size capillaries.

DNA synthesis process

Oligonucleotides were grown in capillaries using standard NPPOC photolabile chemistry (16). NPPOC (Figure 3) was utilized over other photoremovable groups, such as MeN-POC, due in part to its higher 'per synthesis cycle' yield. The LEDs were individually current controlled and run at 20 mA with a National Instruments PCI card (NI-PCI-6704). The DNA sequences were entered into a custom LabView program (National Instruments). This software generated the optimized fluid sequence utilized by the expedite and controlled the on/off pattern of each of the LEDs during DNA synthesis. Through synchronization of fluid delivery and UV light emission (from each LED), discrete species of oligos were grown on the inner surface of the capillary at the location of each LED (Figure 4). A typical DNA synthesis cycle used in this experiment requires 180 s for photodeprotection and 60 s for coupling of the base. Adding other times, this leads to a total of \approx 360 s per base. Hence, the growth of a mixed base 40mer (160 nt) may require up to 16 h. By optimizing the fluid cycle we reduce this time significantly. Further optimization of the pumping process could reduce the time further still.

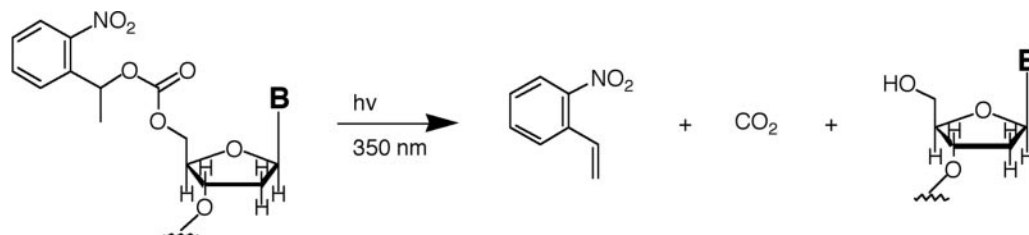


Figure 3. NPPOC chemistry: in the NPPOC deprotection process UV light with wavelength of \sim 350 nm removes the NPPOC protecting group leaving the 5' end ready to couple to the next base.

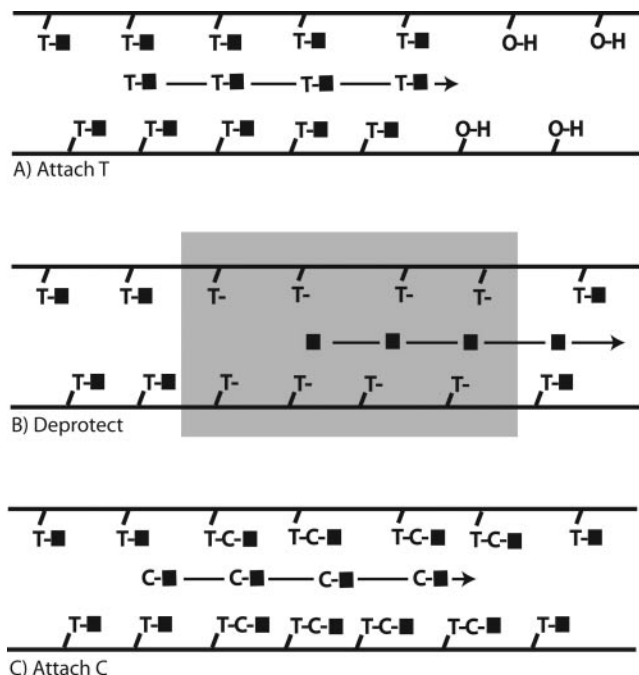


Figure 4. Capillary DNA synthesis: an overview of the DNA synthesis process. A) Step 1: an NPPOC T-amidite (designated by the solid square) flows through the capillary and binds to the free hydroxyl group on the silanized surface of the capillary. B) Step 2: the LED is signaled to emit UV light (shown in grey) releasing the NPPOC protecting group which is then rinsed away. C) Step 3: an NPPOC linked C-amidite flows down the capillary, attaching only where deprotection (loss of amidite protecting group) has occurred. Arrows denote flow direction.

After synthesis the side-protecting groups on the oligonucleotides were removed by immersing the capillary in a 14 ml 1:1 solution of ethylenediamine:ethanol for 2 h at RT. The capillary was then rinsed with distilled water, dried with Ar gas and stored desiccated at RT.

Post-processing operations

After synthesis, several steps are required to characterize the quality of the DNA and to assemble the oligos in longer strands.

Hybridization. To determine the location and identity of the synthesized oligonucleotides, hybridization was performed using 10 μ l of 100 nM Cy3 labeled probe under the conditions described previously (5). The capillary was then visualized using a Nikon fluorescent microscope equipped with MetaMorph imaging software. Because of the geometrical configuration of the capillary, it is difficult to extract quantitative information from the hybridization signal. The images however provide essential information on the location and confinement of the synthesis.

Oligonucleotide characterization and assembly. To characterize the nucleic acid synthesized on the inner surface of the capillary, the oligonucleotides were cleaved from the glass surface and subsequently amplified, sequenced and/or used for gene assembly.

While it is possible to cleave the oligos from the glass surface *in situ*, for this preliminary experiment we removed

the capillary from the fittings and processed it separately. To cleave the DNA off the inner surface of the capillary, a solution of 30% NH_4OH was injected and the solution was incubated for 1 h at RT. The eluate was then collected, dried in speed vacuum centrifuge (240 g) and resuspended in 5 μ l of sterile Milli-Q water.

DNA from an aliquot of the capillary eluate was amplified using flanking 15mer PCR primers containing a MlyI restriction enzyme site (5). After amplification [35 cycles 94°C (30 s), 50°C (30 s) and 72°C (1 min); last step 72°C for 10 min] products were analyzed by agarose gel electrophoresis. Products were then ligated into a Topo-TA blunt vector and transformed into one-shot cells (Invitrogen). Following dideoxysequencing, data analysis was performed using DNASTar software.

Gene assembly was performed as described previously (5). Capillary eluate amplification products were digested with MlyI at 37°C for 1 h. Digested oligos were then assembled via PCR with *Pfu* polymerase, deoxyribonucleotides and buffer [25 cycles; 94°C (30 s), 52°C (30 s) and 72°C (2 min)]. Assembled products were analyzed by agarose gel electrophoresis. Subsequent assembled products were ligated into a Topo-TA blunt vector and transformed into one-shot cells (Invitrogen). Following dideoxysequencing the sequence data analysis was performed using DNASTar software.

RESULTS

We designed three experiments; the first is a fluorescent study of *in situ* hybridization of 25 nt oligomers to map synthesis locations and to show hybridization selectivity. The second is the growth of two different mixed-base 40 nt long sequences in the capillary for subsequent sequencing and assembly. The third is an extension of the second, with the synthesis of a new set of 4 oligos (70 nt) synthesized in two capillaries to be subsequently sequenced and assembled.

Experiment 1: hybridization

To determine if oligonucleotides could be synthesized in a capillary using NPPOC chemistry and LEDs, initial studies focused on growing two species of 25mer mixed base sequences (see Table 1). The sequences chosen had a GC content of 50% and differed by the presence of a single mismatch (MM) (change of A to C) at position 13 to allow for differentiation hybridization to be demonstrated with a single complementary probe.

Hybridization specificity was examined by synthesizing two perfect match sequences flanking a single MM sequence. To grow the two species of oligos within the same capillary, three LEDs (spaced 10 mm apart) were used. After synthesis, a fluorescently (Cy3)-labeled complement was used to identify the location and confirm the identity of the synthesized perfect match DNA.

Imaging of the hybridized capillary under UV illumination revealed three discreet spots along the length of the capillary (Figure 5).

Each of the three hybridized regions matched the location and spacing of the UV-LED light sources of the CSC. Specificity of hybridization was demonstrated, in that the perfect

Table 1. Oligonucleotide sequences synthesized with the CSC

Oligonucleotide	Length (nt)	Sequence(5'→3')
Wt	25	CTGGTCCCACCAAGTACTACTACTG
1 MM	25	CTGGTCCCACCACGTACTACTACTG
OligoA	40	ACATGGAGTCGGTTCAGCCTCAGCGTGTACGACTCGAACG
OligoB	40	ACTCTGAGTCATCGATACGTCGTTTCGAGTCGTACACGCTG
#21	70	CATGGAGTCCGACTTCTCAGAAATGACTTGGTTGAGTACTCACCAGTACAGAAAACACGACTCGTCAT
#22	70	CATGGAGTCCGACTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACACTACGACTCGTCAT
#23	70	CATGGAGTCCGACTAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGACTACGACTCGTCAT
#24	70	CATGGAGTCCGACTCACTCATGGTTATGGCAGCACTGCATAAATCTCTACTGTACTACGACTCGTCAT

Length is denoted by nucleotides (nt).



Figure 5. Comparison of hybridization images: images of sections of the Cy3-hybridized capillary edge (a–c). The central region (b) consists of synthesized target DNA containing a single mismatch whereas the flanking regions (a,c) consist of target perfect match sequences.

match target exhibited a much stronger signal than the target containing a MM. The reduction of fluorescence signal seen between the capillary-synthesized perfect match and single MM oligonucleotides is comparable to the differences seen with similar oligonucleotides synthesized on microarrays utilizing NPPOC chemistry (M. Rodesch, unpublished data).

Only one other report describes the hybridization of oligonucleotides within capillaries (20), and in that case the oligonucleotides were photochemically crosslinked to the inner capillary wall via a photoactive benzophenone derivative. Although hybridization was noted, the hybridization sensitivity of the crosslinked DNA substrate (50 nM Cy3-labeled probe) was much weaker than that seen with our DNA-synthesized substrate (10 nM Cy3-labeled probe). This disparity in sensitivity may be due to a variety of reasons, such as differences in the actual density of oligonucleotides or the DNA availability on the capillary wall.

Experiment 2: gene assembly from capillary synthesized oligonucleotides

To ascertain the quality and biological functionality of capillary-synthesized oligonucleotides, an assembly experiment was designed. Two different 40mers [Oligo A and B (Table 1)] were grown within the same capillary using one LED for each oligonucleotide. Each sequence had a different set of amplification primers such that they could be amplified and analyzed individually. After synthesis the oligos were cleaved from the capillary and PCR amplified.

Each amplification reaction yielded the expected 40mer product, of which a portion underwent sequence analysis or was subsequently digested (to remove the amplification primers) and used for gene assembly. The 60mer that resulted from the joining of the two 40mer was then sequenced.

Sequencing of Oligo A and B 40mer (see Table 2) revealed a level of quality equal to or better than that reported of commercial oligos (21). Although commercial oligos have been

Table 2. Amplified and assembled DNA sequence data

Oligo	#Clones	WT	1MM	2MM	>2MM
A	25	24%	64%	8%	4%
B	20	35%	35%	25%	5%
Product (AB)	18	0%	17%	33%	50%
21,22	42	10%	7%	16%	67%
23,24	45	44%	11%	11%	33%
Product (21–24)	17	6%	23%	23%	47%

Sequences containing no mutations are denoted as wild-type(WT); single mutations as one mismatch(1MM); two mutations as two mismatch (2MM); two mutations or more as (>2MM).

reported to contain 10–25% wild-type sequences [wt; defined as having no (MM)] (21), the capillary synthesized Oligo A and B were comprised of 24 and 35% wt sequences, respectively.

Experiment 3: gene assembly utilizing multiple capillary synthesized oligonucleotides

To assess whether oligonucleotides from multiple capillaries could be combined for applications, such as gene synthesis, sets of overlapping oligonucleotides were synthesized and analyzed pre- and post-assembly. The first capillary grew two 70mer oligos (#21 and #22), and the second capillary grew two additional oligos (#23 and #24) (Table 1).

For amplification, a dilution curve of each of the capillary eluates was tested and products were analyzed by gel electrophoresis. (Figure 6). All dilutions yielded a product of the predicted size (70 nt). Sequencing was performed on the 70mer amplified oligonucleotides from each capillary. The oligos were then digested with a restriction enzyme (MlyI) to allow for release of the primer sequences. The internal 40mer of oligo 21 and 22 contain 20 base overlaps and therefore assembled to form a 60mer product. The internal 40mer of oligos 23 and 24 also contain 20 base overlaps and likewise assembled to form a separate 60mer product. These two 60mer pools were combined to form an assembled 100mer product. Gel electrophoresis was performed to confirm the presence of the 100mer and sequence data was obtained to check product quality (Table 2).

The 70mer amplification products were then processed for subsequent use in gene assembly. The internal 40mer of oligos 21/22 and 23/24 were each assembled to form a 60mer assembly. The two overlapping 60mers were then combined and assembled to form 100mer product. Gel analysis

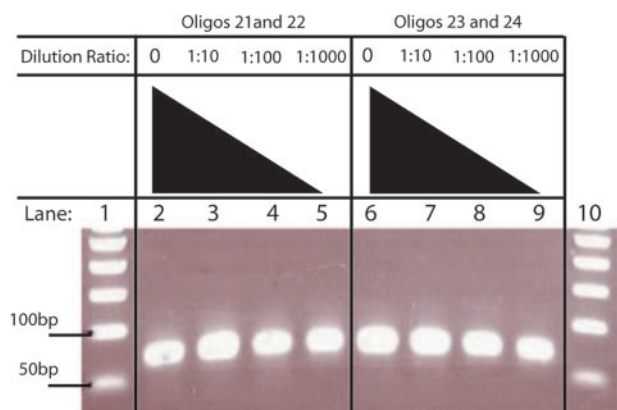


Figure 6. Amplification and analysis of capillary synthesized DNA: PCR amplification products of capillary one (oligonucleotides 21/22) and capillary two (oligonucleotides 23/24) were electrophoresed on a 2% TBE agarose gel, stained ethidium bromide and UV illuminated. Gel (lanes 1 and 10) 50 bp ladder (Stratagene); PCR products of reactions containing capillary one or capillary two eluate with no dilution (lanes 2 and 6), 1:10 dilution (lanes 3 and 7), 1:100 dilution (lanes 4 and 8) and 1:1000 dilution (lanes 5 and 9).

revealed the presence of a 100mer product and sequence analysis confirmed its identity.

Sequence analysis of the amplified products (see Table 2) revealed variability in the performance of the capillaries, with the 21/22 population consisting of 10% wt oligos (7% single MM) and 23/24 having 44% wt oligos (11% single MM). Although the 100 bp assembly product consisted of fewer wild-type sequences than the individual oligonucleotide pools, the overall DNA error rate (mutations per base pair) did decrease by 18–46% (K. Richmond, data not shown).

Sequence analysis of the *in situ* synthesized capillary DNA strands revealed some variability in the quality of DNA synthesized. Although there was a wide range in wild-type population size (3- to 4-fold differences), this span was comparable to that seen in microarray DNA analyses [(21), K. Richmond unpublished data]. Further work is underway to clarify if these differences in DNA quality are due to nucleic acid sequence composition, oligonucleotide length or the synthesis process itself. However, since such a high number of perfect sequences (44% for oligos 23,24) were obtained without post-processing purification, this method is comparable to or surpasses the quality of published commercial oligonucleotides (21).

SUMMARY AND CONCLUSIONS

We present the first report of synthesis of DNA within capillaries through utilization of LED optics and photo-labile chemistry. While *in vitro* DNA synthesis is well established, specific chemistry and platforms for synthesis continue to evolve. In recent years, a variety of chemistries have been employed to synthesize DNA on surfaces ('DNA chips') with the specific goal of achieving a large number of sequences per chip. These include inkjet delivery systems with standard DMT protected phosphoramidite chemistries (Agilent), deprotection strategies including photogenerated acid (2), electronically generated acid (4) and those employing

direct light mediated removal of the protecting groups MeNPOC (3) and NPPOC (5,18). These methods have been used with different platforms to produce microarrays for gene expression and tiling, gene assembly (5,6) and other assays (ChIP-chip etc.). However, these platforms generally require costly elements (physical masks) and/or complex apparatus (fluidics, optics and light sources), thereby making them cost-prohibitive for the average laboratory.

Use of the CSC allows for the synthesis of multiple oligonucleotides within a single capillary, effectively multiplexing a single-product synthesizer. Growth of multiple DNA sequences allows for a more efficient use of synthesis reagents and maximizes the productivity of the system. The production of oligos was performed in a controlled and addressable manner within the capillary as confirmed by hybridization and re-sequencing. Previous work (20) produced capillaries with DNA covalently bound to the surface, and demonstrated similar strong binding results when hybridization to perfect sequences was performed. Unfortunately, no hybridization control was done while using a target containing a single MM, so it is unclear if the hybridization differentiation of this prior method is as sensitive as that seen with our system. Using either of these two methods, the DNA-capillary product could be used for any number of biological applications, such as SNP detection, DNA protein binding studies or genetic screening. However, use of the covalent attachment method requires separate synthesis of each of the DNA oligos to be used before attachment to the capillary, thereby increasing cost and decreasing system flexibility.

In addition to using an optimized fluid cycle and small volume of DNA synthesis reagents, the CSC can be modified to allow for high throughput applications. To increase the number of different sequences synthesized we can utilize longer capillaries (able to accommodate a larger number of LEDs) or a set of shorter capillaries connected in parallel, possibly with manifold valves to direct the reagent flow. Due to the cost of the reagents, it is essential to keep the volume as small as possible. Thus longer capillaries will require a smaller diameter. Smaller capillaries, such as drawn silica glass capillaries with an outer diameter of 360 μm and inner diameter of 100 μm and coated with a UV transparent polymer (Polymicro Technologies) are one solution. The small inner diameter of these capillaries would allow for a capillary 5 m long with a volume of only 40 μl , about the same volume of the capillaries reported in this paper! As an example, a 100 LED system could be assembled using a 1m capillary if we consider a conservative 1 cm spacing between the LED sources. This system could produce 160 nt in ~ 16 h, or 240 nt/day. The quantity of oligos is dependent on the area illuminated by the LEDs, on the order of 0.063 cm^2 (i.e. ≈ 1.2 pmol per sequence) for the current CSC. This synthesis operation would require approximately \$272 at today's reagent costs. Figure 7 shows the pressure required to pump the reagents through capillaries of different size and length and looks at a comparison of different fluid volumes. While fluid delivery to larger capillaries can be supplied by any standard DNA synthesis fluidics system, a high-pressure fluid delivery system is needed to move liquids through smaller capillaries. If warranted, bead-loaded capillaries could also be used to increase the yield of oligos. For lower

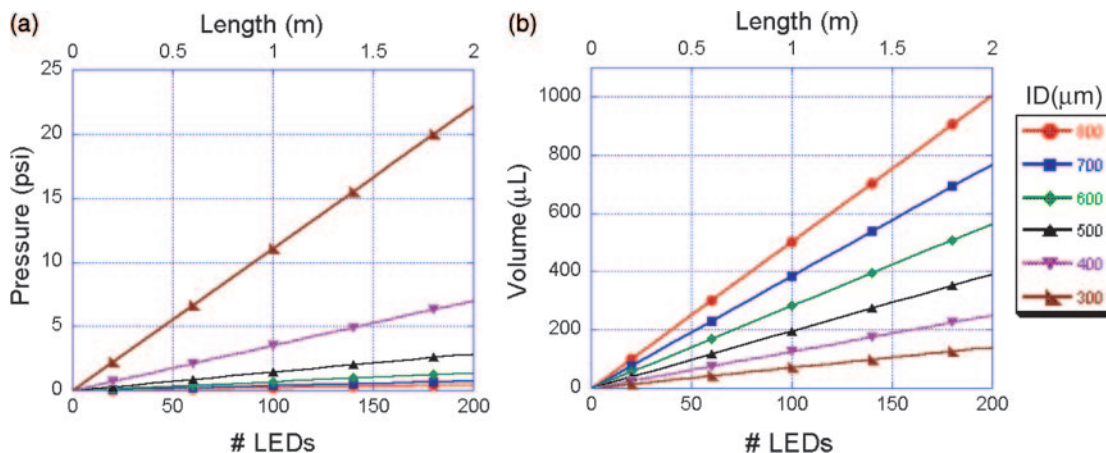


Figure 7. CSC scalability calculations: analysis of the effect that capillary diameter has with varying LED population sizes. Parameters examined are (a) fluid pressure and (b) total reagent volume.

throughput applications, the reported CSC may be applicable as it has been used to synthesize three unique oligo species per capillary (S. Blair, unpublished data), with a maximum number of oligo species still to be determined. The flexibility of the design allows for multiple solutions. For instance, arranging several short small inner diameter capillaries in parallel with LEDs arrayed along their lengths would allow the simultaneous production of a large number of oligos in a small bench top device.

Collaboration between the fields of engineering, chemistry and biology are crucial for continuing advances in the field of molecular biology. As nanotechnology and miniaturization become the hallmark of engineering and biological scientific endeavors, the need for small quantities of diverse populations of DNA oligonucleotides is growing, particularly in the area of gene synthesis. By combining off-the-shelf materials and principles from these varied fields, a simple and reliable capillary synthesis cell has been created as a cost-effective system for high quality DNA synthesis.

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Conflict of interest statement. None declared.

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