Rapid On-Demand Enzymatic DNA Synthesis (EDS) Sets a New Standard for Access to High-quality DNA Oligos

Phosphoramidite-based processes have been the gold standard for DNA synthesis for over 40 years, despite unmet challenges related to broad-based, reliable access, environmental sustainability, the high cost of modifications required for many standard applications, and the length of high-quality oligos that can be produced with standard processes. Within a few years, DNA Script's novel EDS process has largely overcome these challenges, offering consistent, on-demand production of high-quality oligos for a broad range of applications in a standard laboratory environment, with full control over delivery times and sequence information.

INTRODUCTION

Oligonucleotides are ubiquitously used in molecular and synthetic biology, omics applications, and diagnostic assays—to target, barcode, label, connect, modify, and build biological molecules; and activate, inhibit, or modulate biochemical reactions. For the past four decades, these primers, probes, adapters, gene fragments, and other nucleic acid-based tools have been synthesized almost exclusively by a handful of large, centralized commercial oligo manufacturers, using a chemical phosphoramidite solid-phase synthesis process[1,2] (Figure 1).

CDS vs. EDS PROCESS

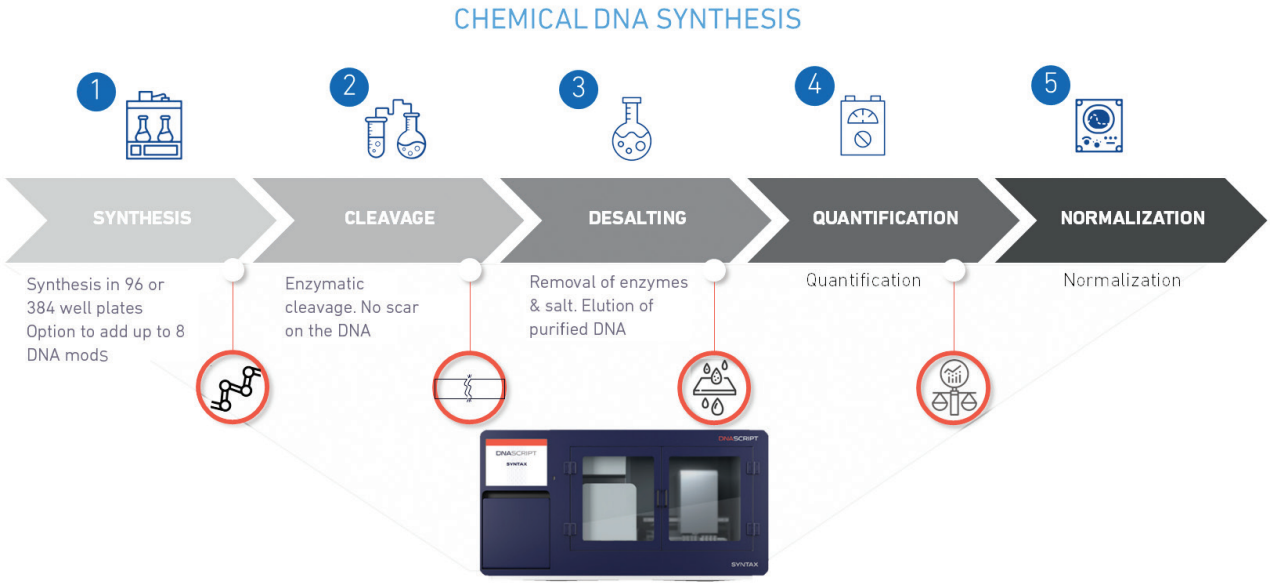

FIGURE 1.
Chemical DNA synthesis (CDS, top), more commonly known as phosphoramidite solid-phase oligonucleotide synthesis, is typically achieved via five steps and seven distinct chemical reactions: (i) protonation; (ii) n cycles of (detritylation, tetrazole activation and coupling, capping of unreacted nucleotides on a resin, oxidation, and detritylation), (iii) cleavage from the solid support, and (iv) deprotection (see Hoose et al.2for a detailed diagram). After synthesis, additional purification and analysis processes and equipment are needed to prepare ready-to-use oligos. The EDS process developed at DNA Script (bottom) consists of repetitive synthesis cycles comprised of (i) elongation (addition of a single, reversibly protected standard or modified (e.g., dye-labelled) nucleotide to an initiator DNA), and (ii) deprotection (to allow incorporation of the next base).3 Synthesis, as well as post-synthesis processing (cleavage from the initiator DNA, desalting, quantification, and normalization) are all performed intervention-free in plate-based format using a single SYNTAX instrument. The SYNTAX platform currently produces >200 pmol of ready-to-use, scarless DNA (normalized to up to 10 µM) per well, in as little as 4 hours (≤60-mers) or overnight (61- to 120-mers). In contrast, CDS takes 13 – 30 hours and requires specialized and separate equipment for synthesis, cleavage, desalting, quantification, and normalization.

Despite being the industry standard, chemical DNA synthesis (CDS) suffers from inherent and unaddressed challenges that are exacerbated when long and chemically modified oligos are needed (Table 1).

DNA Script has developed a novel, fully enzymatic DNA synthesis (EDS) process and benchtop SYNTAX™ platform that overcomes the limitations of CDS and sets a new standard for access to high-quality DNA oligos up to 120 nt in length (Figure 1).3

In this study, we have compared DNA oligos (60- and 120-mers) sourced from four leading commercial suppliers (CDS oligos) with those produced in-house using the SYNTAX platform (EDS oligos), with respect to three key metrics: delivery time, quality (purity and sequence accuracy) and consistency. EDS significantly outperformed CDS with respect to delivery time and batch-to-batch consistency, with comparable or improved sequence accuracy.

TABLE 1. KEY DIFFERENCES BETWEEN CHEMICAL AND ENZYMATIC DNA SYNTHESIS PROCESSES

	Chemical DNA Synthesis (CDS)	Enzymatic DNA Synthesis (EDS)
Accessibility	• Requires harsh chemicals, specialized infrastructure, and expertise. Commercial supply is dependent on a handful of large, centralized manufacturers.	• EDS oligos can be produced by entry-level technicians in a standard laboratory environment using the SYNTAX Platform.
Environmental impact	• Produces 8-fold the amount of liquid waste compared to EDS, 100% of which is hazardous.[4]	• 80% of waste is aqueous.
Confidentiality	• Subject to third-party supply chain and cybersecurity risks, logistical challenges, and global events.	• Oligos are synthesized in-house without the need to share sequence information.
Turnaround time	• Ranges from overnight to several weeks (dependent on oligo length, complexity, and geographical location).	• Same- or next-day synthesis of oligos up to 120 nt or with chemical modifications (e.g., fluorescently labeled probes).
Efficiency	• Despite four decades of improvement, elongation cycle efficiency limits oligo purity and length to <300 nt. Some of the sequences cannot be produced.	• Cycle efficiencies of 99.6% and synthesis of challenging motifs are achievable via biomolecular engineering. Synthesis of difficult and very long (>300 nt) DNA fragments has already been demonstrated.[4]
Additional benefits	• The vast majority of oligos are synthesized in the 3' → 5' direction and carry a 5'-hydroxyl group. Phosphorylation for ligation-based workflows incurs an additional charge.	• Oligos synthesized in the "natural" 5' → 3' direction, with a default 5'-phosphate.

STUDY DESIGN

This study was performed using oligos of two different lengths:

- **60-mers**, the average maximum length that can be requested for regular, affordable CDS oligos from commercial suppliers
- **120-mers**, the maximum length supported commercially on the SYNTAX™ platform with the latest version of the DNA Script EDS chemistry.

For each length, a set of sixteen standard oligos (comprised of unmodified dA, dC, dG, and dT; see Appendix) was selected from a pool of randomly generated sequences to represent a broad range of sequence diversity. Each set included several oligos with challenging codons or motifs (such as hairpins or G-quadruplexes).

The oligo acquisition scheme is outlined in Figure 2. Unless indicated otherwise, oligos were prepared using standard synthesis methods, purified by desalting, constituted in nuclease-free water, and delivered in 96-well plates.Oligos were evaluated based on three key criteria:

1. Delivery time
2. Oligo purity
3. Sequence accuracy

Criteria are defined in Table 2, whereas analysis methods are described in Materials & Methods.

OLIGO ACQUISITION SCHEME

	Supplier A	Supplier B	Supplier C	Supplier D	SYNTAX Platform
60-mers (*n*=16)					
Number of orders	8	8	8	8	8
Synthesis method	Standard	Standard	Standard	Standard	Standard
Purification method	Standard desalted	Standard desalted	Standard desalted	Standard desalted	Standard desalted
Oligos delivered in	96-well plates	96-well plates	96-well plates	96-well plates	96-well plates
120-mers (*n*=16)					
Number of orders	4	4	4	4	4
Synthesis method	*Specialized*	Standard	Standard	Standard	Standard
Purification method	*Specialized*	Standard desalted	Standard desalted	*PAGE*	Standard desalted
Oligos delivered in	96-well plates	96-well plates	96-well plates	*Tubes*	96-well plates

FIGURE 2.
CDS oligos were ordered from four leading commercial suppliers (A, B, C, and D) for delivery to Paris, France. All orders were separately placed, fulfilled, and delivered. EDS oligos were printed in-house as described in Materials & Methods. One independent EDS run was completed for each of the eight (60-mer) or four (120-mer) commercial orders. PAGE: polyacrylamide gel electrophoresis.

TABLE 2. KEY METRICS ASSESSED IN THIS STUDY

Delivery time	Elapsed time (in days) from the day on which an order was placed with a commercial supplier (for CDS oligos) or a run was configured (for EDS oligos) to the day on which oligos were delivered or ready for use.
Oligo purity (%)	Proportion of oligos in a preparation that have the expected length. In this study, determined by capillary electrophoresis (for 60-mers only, see Materials & Methods for details).
Oligo sequence error	Nucleotide at any position in an oligo that does not correspond with the expected sequence. The error may be an insertion, deletion, or substitution.
Per-position error rate (%)	Assessed by deep sequencing on the Illumina® platform (see Materials & Methods). Number of errors at a specific position normalized against per-position sequencing depth.
Overall error rate (%)	(Sum of per-position error rates) ÷ (oligo length in nt)
Sequence accuracy (%)	100% (Overall error rate)
Consistency	For any metric, assessed by means of the standard deviation across all data points.

RESULTS & DISCUSSION

Delivery time

The average time from placing an order with a commercial supplier to receipt at the DNA Script facility in Paris was 9 ±5 days and 18 ±11 days for 60- and 120-mers, respectively (Table 3). Delivery times for individual orders varied significantly and were impacted by the following factors:

- Slow ordering processes (need for multiple e-mail exchanges before an order could be placed),
- Production or delivery delays
- Lost packages (entire order had to be resynthesized).

In addition, oligo plates or tubes were not always clearly labeled or were mislabeled, resulting in the need to resynthesize orders.

In contrast, EDS oligos were consistently produced in 0.5 or 1 standard workday for 60- and 120-mers, respectively—from run configuration using the SYNTAX Console Software to availability of a 96-well plate of ready-to-use oligos. The SYNTAX platform further facilitates the rapid availability of oligos for downstream use by producing a full run report (oligo sequences, concentrations, and final volumes) in comma-delimited format.

TABLE 3. OLIGO DELIVERY STATISTICS

	CDS oligos					
	Supplier A	Supplier B	Supplier C	Supplier D	All Suppliers	EDS oligos
Mean delivery time for 60-mers (*n*=8)	8 ±3 days	6 ±2 days	11 ±7 days	11 ±4 days	9 ±5 days	0.5 ±0 days
Mean delivery time for 120-mers (*n*=4)	14 ±10 days	6 ±1 days	19 ±4 days	30 ±5 days	18 ± 1 days	1.0 ±0 days

Purity

Irrespective of whether oligos are synthesized chemically or enzymatically, the efficiency with which a single nucleotide is added in each cycle has a large cumulative impact on the yield of full-length products (purity; see Figure 3). Theoretical yield may be calculated using the formula:

$$Yield = (ECE)^{n-1}$$

where *ECE* is the effective coupling efficiency and *n* is the oligo length.

IMPACT OF PER-CYCLE COUPLING EFFICIENCY ON OLIGO PURITY

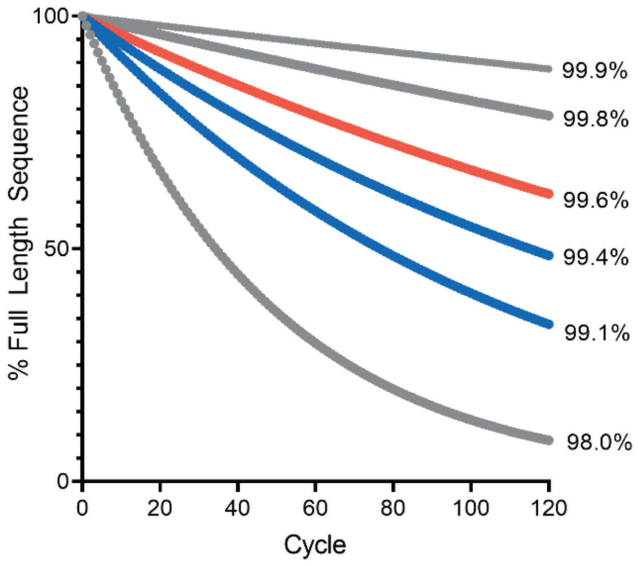

FIGURE 3.
Theoretical % of full-length molecules for oligos up to 120 nt, calculated for per-cycle coupling efficiencies ranging from 98.0 – 99.9% (grey). The DNA Script EDS process routinely achieves an average per-cycle coupling efficiency of 99.6% (red), compared to 99.1% - 99.4% for CDS (blue) – which translates to a theoretical oligo purity for 60-mers of 79.0 %. This is equivalent to the purity achievable for 27-mers with the industry average coupling efficiency of 99.1%.[5]

For example, the synthesis of a 60-mer with a coupling efficiency of 99.0% will theoretically yield $(0.99)^{59}$ = 55.3% full-length oligos. Recursive impacts of partial losses with every cycle may be offset by:

- Purification using techniques such as polyacrylamide gel electrophoresis (PAGE) or high-performance liquid chromatography (HPLC), which effectively excludes shorter oligos, but inevitably results in asignificant loss of material (as compared to standard desalting) and adds cost, or

- Continually improving the average coupling efficiency of enzymatic fragmentation (through biomolecular engineering) beyond 99.4%, which appears to be the upper limit for chemical synthesis.[5]

Empirical determination of oligo purity is not trivial. We have developed a method employing capillary electrophoresis that can be used to determine the purity up to 60 nt in length (see Materials & Methods for details). Results for all of the 60-mers included in this study (all purified using standard desalting) are given in Figure 4. The purity of EDS oligos (73.7 ±2.1%) was comparable to the mean purity of the four commercial suppliers (71.7 ±7.1%), but variation across orders (EDS runs) was markedly smaller than for different batches of CDS oligos.

AVERAGE PURITY OF 60-MERS USED IN THIS STUDY

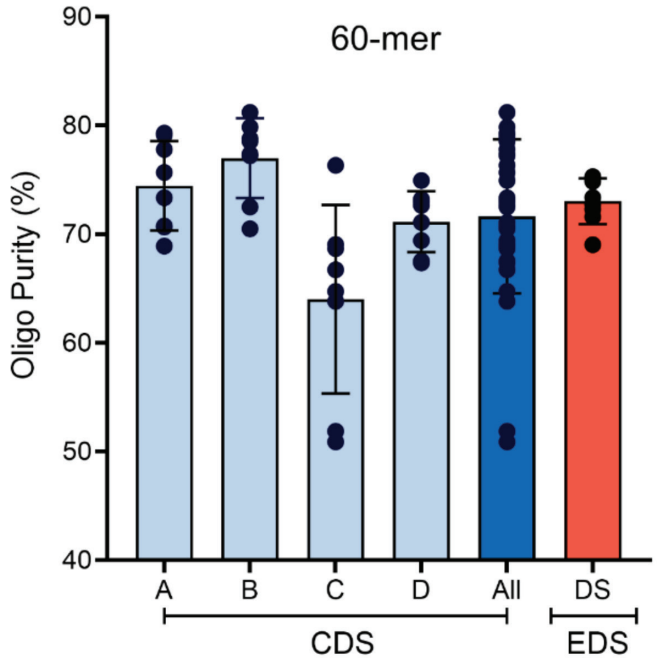

FIGURE 4.
Data represent the average purity of the sixteen 60-mers for each of the eight separate orders/EDS runs, which was 74.4 ±4.1% (Supplier A), 77.0 ±3.7% (Supplier B), 64.0 ±8.7% (Supplier C), 71.1 ±2.8% (Supplier D), 71.7 ±7.1% (all CDS suppliers), and 73.0 ±2.1% for DNA Script (DS, EDS oligos), respectively.

Sequence accuracy

Oligo users typically have a good understanding of the potential impact of low oligo purity (incomplete sequences) in their application and will pay more for an appropriate purification method to remove these if necessary. Nevertheless, the fact that the full-length oligos in preparation are not all sequence-perfect is not often contemplated. For most applications, this does not matter, as the
outcome of an assay is determined by the **average** sequence at each position in the preparation (which corresponds to the desired nucleotide in

>99% of cases). However, synthesis errors can significantly impact applications where individual copies of an oligo are propagated or interrogated including those involving cloning, gene assembly, gene editing, next-generation sequencing, and data storage.

At DNA Script, we routinely use deep sequencing on the Illumina® platform to assess the error rate of our EDS process and track improvements. Of the different types of errors:

- **Substitutions** are extremely rare but can occur when the unused nucleotide from a previous cycle was not fully removed, or individual nucleotides are not 100% pure.
- **Insertions** are also extremely rare and are most likely the result of imperfections in reversibly terminated nucleotides, leading to the addition of two nucleotides (typically of the same kind) in a single cycle.
- **Deletions** are the most common error (in both EDS and CDS), and result from incomplete incorporation of the desired nucleotide in each synthesis cycle. Note that, unlike CDS, capping is not employed in EDS to reduce the accumulation of deletion mutations.

Mean error rates for 60- and 120-mers included in this study are given in Figure 5A and 5B, respectively. Compared to CDS oligos, the mean error rate for EDS oligos was:

- Comparable to the average performance of commercial supplier(s) for 60-mers.
- Better than three of four commercial suppliers for 120-mers. Keep in mind that Supplier A uses a specialized process for long oligos and that oligos from Supplier D were subjected to an expensive PAGE purification process.
- Less variable across separate synthesis events, particularly for 120-mers.

MEAN ERROR RATES

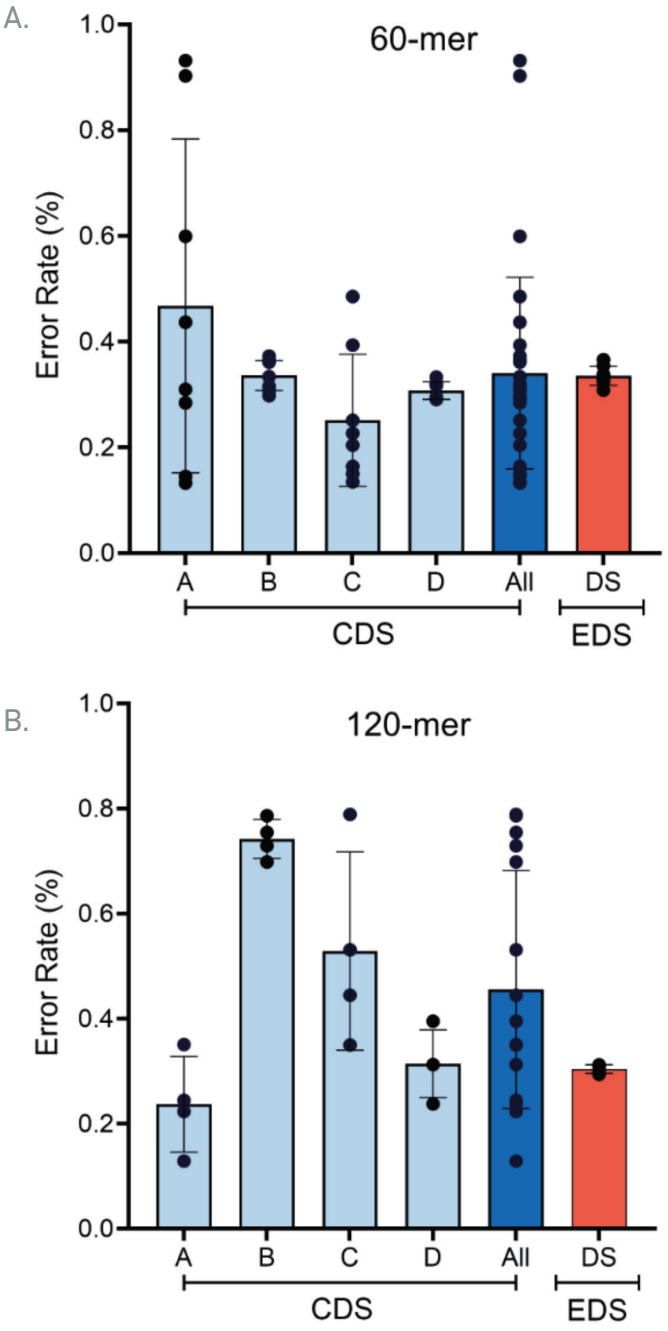

FIGURE 5.
Data represent the mean error rate for (A) the sixteen 60-mers produced across eight distinct orders/runs, and (B) the sixteen 120-mers produced in four distinct orders/runs. For 60-mers, values were 0.46 ±0.32%, 0.34 ±0.028%, 0.25 ±0.12%, 0.31 ±0.017%, 0.34 ±0.18%, and 0.34 ±0.018% for the four commercial CDS oligo suppliers (A – D), all commercial suppliers combined (All), and DNA Script (DS, EDS oligos), respectively. Corresponding values for 120-mers were 0.24 ±0.09%, 0.74 ±0.037%, 0.53 ±0.19%, 0.31 ±0.064%, 0.46 ±0.23%, and 0.30 ±0.008%.

CONCLUSION

Oligos are an "everyday" reagent in life science laboratories. Users who require a few standard PCR primers per week and are located within a two-day delivery radius from an established commercial supplier hardly have to to give oligo ordering any thought. However, when an experiment or workflow calls for large numbers of oligos, oligos with modified bases, or long oligos that require a specialized synthesis process and/or additional purification, planning, and budgeting are no longer trivial. As shown here, delivery times from commercial suppliers of <1 week are the exception rather than the rule and are subject to a multitude of potential delays that impact productivity and control over project timelines.

DNA Script's Enzymatic DNA Synthesis technology (EDS) and benchtop SYNTAX platform enable rapid, on-demand production of DNA oligos (up to 120 nt in length and in batches of ≤96) in any molecular biology laboratory. Projects may be queued and printed as soon as sequences have been confirmed, making ready-to-use primers, probes, or other oligos available in ≤24 hours, irrespective of oligo length or design.

With impediments to access removed, the key question is whether EDS oligos support the same levels of performance as oligos produced with tried-and-trusted chemical synthesis (CDS). This study provides conceptual proof that this is the case, by demonstrating equivalent or higher EDS cycle efficiency, oligo purity, and sequence accuracy than that available from industry-leading CDS oligo suppliers. In addition, the consistency of EDS oligo quality across different synthesis batches was shown to be significantly better than that of CDS oligos sourced from commercial suppliers.

In addition, EDS oligos have been shown to perform equivalently to or better than CDS oligos across a wide variety of applications. Evidence of the functional performance of EDS oligos in standard PCR, SYBR Green-based qPCR, TaqMan assays, targeted sequencing (hybridization capture- or amplicon-based), gene assembly and cloning, FISH, CRISPR guide RNA construction, DNA storage, and many other applications may be found in an ever-growing collection of Application Notes and peer-reviewed publications produced by early adopters of EDS technology.[6,7]

MATERIALS & METHODS

Enzymatic DNA Synthesis

EDS oligos were printed in-house at DNA Script (Paris, France) on a SYNTAX Model STX-200 Platform (cat. no. 101773). All oligos were produced with the SYNTAX DNA Synthesis Kit and SYNTAX 96 Hi-Fidelity Run Kit (cat. nos. 101867 and 101868).

Purity Assessment

Oligos (60-mers only) were analyzed using an Oligo Pro II System (Agilent® Technologies). This system employs capillary electrophoresis and UV spectroscopy (260 nm) to enable separation and detection of single- or double-stranded DNA oligonucleotides up to 60 nt/bp in length at single-base resolution. The fraction of 60-mer in each CDS/EDS preparation was calculated using the Agilent Oligo Pro II Data Analysis Software Revision 2.0.0.3.

Calculation of Error Rate

An aliquot of every oligo from every CDS order/EDS run (16 x 8 x 5 for 60-mers and 16 x 4 x 5 for 120-mers) was converted to an indexed library for Illumina® sequencing using the xGen™ ssDNA & Low Input DNA Library Prep Kit and UDI Primers (Integrated DNA Technologies).
Libraries were pooled and subjected to paired-end sequencing (2 x 75 bp for 60-mers and 2x151bp for 120-mers) on an Illumina MiSeq® instrument using MiSeq® Reagent Kit v2. Pools were configured to achieve a minimum of 10 000 reads per sequence.

Adapter trimming was performed using trimmomatic, forward and reverse reads were joined using bbmerge, and alignment to theoretical sequences (see tables A1 and A2 in the Appendix) was performed using bowtie2. At each position of a given reference sequence, non-reference bases were categorized as insertions, deletions, or substitutions. For each position, the per-position error rate was calculated by dividing the number of reads with errors by the per-position sequencing depth. Per-position error rates are then averaged across the length of the reference to calculate the overall error rate for each oligo.

Statistical Analysis

Statistical analysis was performed, and graphs were generated using GraphPad Prism (version 9.3.1).

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APPENDIX MATERIALS & METHODS: OLIGO SEQUENCES

TABLE A1. 60-MERS

Oligo ID	Sequence (5' → 3')
1_60	ACGACCTACAGAACAAACCGGGGTTCCGAGCGGTAATAGCAACACCAACGAGATATAAGA
2_60	GTCTCTGCGGAGGAAGACACTTCGGCTTCGCGGAATCGACTATCAGGCGGTCGCCACAGA
3_60	GACTTATCCCCCAAGGATTTGTACGTGACTCCTTATAAGGTATGTCGTGCCGATCCACGA
4_60	TAGTAGCAAGAGCACCTCCTTCTAGTCTTTACCCGACTGATAACCGCGACCTAGCATCGA
5_60	ATCAATCCTAGTACATCCGCGATAGGATTTCTAGTTACTATATCATCAGCTGGAAGTGGA
6_60	CTTGGGACTACCCAACAGCGTTGAGTGATCATACACAGGTATTCCAGCGATGGCATCCGA
7_60	GTATGGCGCGATGACTCGCGCACGCTACGGATTCACTTGCTAAATATCACCTTTAACTGA
8_60	AGTTAGTCAAAACCATGTCCTACCTAAGTAAACCTCCCAAGGAAGAAACAGTAGATGAGA
9_60	ACTGGAGAGAAGCTATGTATATATATCGGCCGCGTCATGCTGCGTTGCACTCAGTGCCGA
10_60	TCTGTGTCGCGCCCTCGTAACTGCGTTGTTTATTTTGTCGAGATAACGTTGGGCCTCAGA
11_60	AGGGGAATAACCCATTTTTTCTTGCAACCAGAATGTGGTTGCCTATACTGTACCTCCTGA
12_60	TGATGGCGGTTCAGGCACACAGGGGTCCGTGCGGTTCCGCACAGGGCAATGCATCCATGA
13_60	TGGCGCTGTTTCGCGTGACATTCTAAATACGGATGTGGCATCCGACTGGGATTCGAGTGA
14_60	AGGCCTGTCCTATGGCTAGGACTCTGGTCACTGCAAAGGGAAAAGGCAACTTGTGGCAGA
15_60	GCATGAACCTTTCGACATCTAACCTTTACCCCAATTCCGTCTAGGCCTCAAAGTGTAGGA
16_60	GATTGCGGGCGATGGGTGAGGCTAAGCCAGCGATACCTTGTCAATGACTTGTTCTTGTGA

Codons highlighted in red are regarded as difficult-to-synthesize with chemical and enzymatic processes. Nucleotides highlighted in blue represent motifs that could potentially participate in the formation of G-quadruplexes.

APPENDIX MATERIALS & METHODS: OLIGO SEQUENCES

TABLE A2. 120-MERS

Oligo ID	Sequence (5' → 3')
1_120	ACGACCTACAGAACAAACCGGGGTTCCGAGCGGTAATAGCAACACCAACGAGATATAAGACGTACT ATAGATCATAAAGTGATATTGATCTCGGTCCCGACAGTCGCGAACAGTTCAAGA
2_120	GTCTCTGCGGAGGAAGACACTTCGGCTTCGCGGAATCGACTATCAGGCGGTCGCCACAGAGCTTAG ACTCCTAGCCAGTAGTTTCATCGGCTATGATAGAAACCCTAAGGACTCTTCAGA
3_120	GACTTATCCCCCAAGGATTTGTACGTGACTCCTTATAAGGTATGTCGTGCCGATCCACGAGCAAAG AGCGTCCTTTGACGTCTAACTACCCTTGGATAGACTTAACATCTAAAAGCGCGA
4_120	TAGTAGCAAGAGCACCTCCTTCTAGTCTTTACCCGACTGATAACCGCGACCTAGCATCGAATTTCC GTACAGCTTTATTTACCGCTAAGGATGTAGGAGAGAAGTTCTATGGGGACCCGA
5_120	ATCAATCCTAGTACATCCGCGATAGGATTTCTAGTTACTATATCATCAGCTGGAAGTGGAAAGCGC GCGAGAATTGTAGCTACGGAACGCCGTAAAGTGACGCGGCGTGGCCGGCTTTGA
6_120	CTTGGGACTACCCAACAGCGTTGAGTGATCATACACAGGTATTCCAGCGATGGCATCCGACCTAAA ACTGCCGGATCAGTTGGACTGCGAAGAATCCCGAACAATTGTCTTCGTAGTGGA
7_120	GTATGGCGCGATGACTCGCGCACGCTACGGATTCACTTGCTAAATATCACCTTTAACTGAGGCTAT TACGGAAGGGCGGTATGTAATGTGTACAAGTTGATCAACGCGATTCTCGTGCGA
8_120	AGTTAGTCAAAACCATGTCCTACCTAAGTAAACCTCCCAAGGAAGAAACAGTAGATGAGATTACGT GCGAGCTACCAGACATAAGGAGCCGATTTAAGGAATTTAGAAATTGAGTGATGA
9_120	ACTGGAGAGAAGCTATGTATATATATCGGCCGCGTCATGCTGCGTTGCACTCAGTGCCGATATCTA CCCCCTTATGAGACGAGAACGCTTGTGCCGATGACTGGGGCGATGTGGCCAAGA
10_120	TCTGTGTCGCGCCCTCGTAACTGCGTTGTTTATTTTGTCGAGATAACGTTGGGCCTCAGAGGCTAG GTGGGTCTGTAAAAGAACATGAGACCCTTGTTGAAAGTAGGAGCTGGACGTAGA
11_120	AGGGGAATAACCCATTTTTTCTTGCAACCAGAATGTGGTTGCCTATACTGTACCTCCTGATGTATA TCCCACTAAGAACCTGGTACGTGCCTTTCCGGGACGTGCGGACCCAGACCCAGA
12_120	TGATGGCGGTTCAGGCACACAGGGGTCCGTGCGGTTCCGCACAGGGCAATGCATCCATGAGGTTAG TACCGATGGGGTAAGTACTAAAGATACGCCGAGAGCGTCTATATTGTAGACTGA
13_120	TGGCGCTGTTTCGCGTGACATTCTAAATACGGATGTGGCATCCGACTGGGATTCGAGTGAGCATTC ACGTTTCAACGGGTGCACCAAAGACTAATATTGACGGCTGTTTAGGTATCTGGA
14_120	AGGCCTGTCCTATGGCTAGGACTCTGGTCACTGCAAAGGGAAAAGGCACTTGTGGCAGATCGGCA TATGGTCTCTCGAGTCATGTGTGTCAGATGCTGACCGACTAAATACCCACTTGA
15_120	GCATGAACCTTTCGACATCTAACCTTTACCCCAATTCCGTCTAGGCCTCAAAGTGTAGGAGGCTAG TCTCTGCGACAAAGTGAGGACATGCCTACACAGACGGACCGGTAGTGGGGTAGA
16_120	GATTGCGGGCGATGGGTGAGGCTAAGCCAGCGATACCTTGTCAATGACTTGTTCTTGTGAGCATCA CGTACTACTGCATAGATAGGGTTGACTACCAAGATACTATGCATATAAGAGAGA

Codons highlighted in red are regarded as difficult-to-synthesize with chemical and enzymatic processes. Nucleotides highlighted in blue represent motifs that could potentially participate in the formation of G-quadruplexes.

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