Building genes from scratch: A cost and time assessment of novel enzymatic DNA synthesis technology

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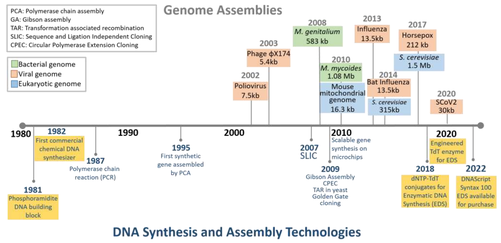
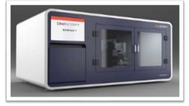
INTRODUCTION



- **New technologies expand our ability to create and assemble complex DNA sequences, even 1Mb genomes (above).**
- **Phosphoramidite chemical DNA synthesis** has been the standard method since 1980's and is used widely by commercial vendors (IDT, Genscript, Eurofins, etc.).
- **Enzymatic DNA synthesis** has recently emerged as a viable competitor in the synthetic DNA market, replacing the hazardous organic waste stream with a more user-friendly platform and aqueous waste stream.

Chemical DNA Synthesizer MerMade 192e, LGC solutions



Enzymatic DNA Synthesizer Syntax 100, DNAScript

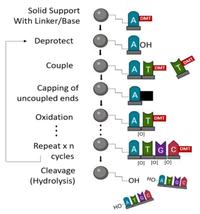
- **Here we evaluate novel enzymatic DNA synthesis technology (Syntax 100) and compare it to commercially chemically synthesized DNA (IDT) to create synthetic oligonucleotides and assemble the gene encoding Green Fluorescent Protein (GFP).** In this assessment we consider cost, time of assembly and precision/accuracy of sequences using each technology.

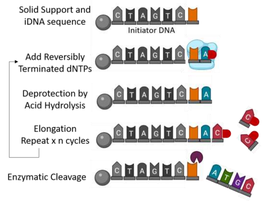
METHODS

How Does Enzymatic DNA Synthesis Work?

The first to market enzymatic 'DNA Printer' from DNAScript, the Syntax-100 uses an **engineered Terminal Deoxynucleotidyl Transferase (TdT)** enzyme to add **reversibly terminated nucleotides** to the **3' end of a DNA strand**, allowing tight control over each step of synthesis (fewer steps than chemical DNA synthesis shown below).

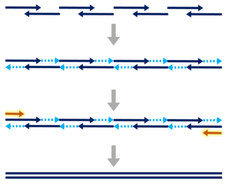
Chemical synthesis (MerMade)	Enzymatic synthesis (Syntax)
192 x 20mer in 18hr, 3'-5' direction	96 x 60mer in 13hr, 5'-3' direction
100-300 bp max	80 bp max
Hazardous organic waste (~5-8L/run)	Acidic aqueous waste (~1-2L/run)
0.1-1% error rate	0.4% error rate
Requires cleanup post-synthesis	Cleanup included in run time





How Do You Build A Gene?

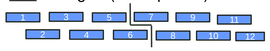
1. Design and synthesize overlapping oligonucleotides
2. Assembly overlapping oligos through PCR (PCA)
3. Enrich final product using end oligos and PCR
4. Clone into a plasmid
5. Confirm by sequencing



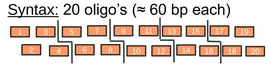
EXPERIMENTAL SETUP AND RESULTS

Aim 1. Design and synthesize overlapping oligos for GFP sequence (Syntax vs IDT)

IDT: 12 oligo's (≈ 90 bp each)



Syntax: 20 oligo's (≈ 90 bp each)



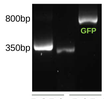
GFP (717 bp)

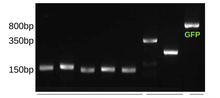
Printing DNA on Syntax-100

- 15 min setup to upload sequences and load reagents
- Software flags potentially difficult sequences (top right), user can edit before the run
- End of run (13hr for 96x60mers) oligos are cleaned, quantitated and normalized (max is 7 µM) ready to use for downstream applications (bottom right)
- **We produced 2 plates of 96 oligos/plate in 2 days (3 sets of GFP oligos including duplicates,additional end and cloning oligos; note: Syntax GFP sets 2 and 3 were produced on the same plate**

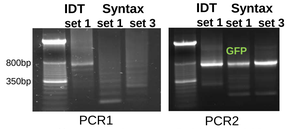
Aim 2. Assemble GFP sequences using PCR

Method 1: Subpool and PCA





Method 2: 1-step assembly and PCA



1-step isothermal assembly of oligos decreased overall number of PCR rounds for Syntax oligos and also increased number of oligos in pool (20 vs 6)

Aim 3. Clone full length GFP sequences into plasmids and analyze by sequencing/protein expression

1. Final PCR products were Topo-cloned and screened for correct inserts before sequencing (Eurofins)
2. Perfect sequences we cloned into a pET28 vector using Gibson assembly
3. We compared IDT oligos and Syntax oligo assembled GFP sequences for cost, time, and sequence accuracy, and protein expression in *E. coli* (not pictured here)

Sequencing results

Oligo source	Assembly method	# pos/screened	# sequenced	perfect	1 bp SNP/ indel	large indel(s)/ 2+ SNPs
IDT Set 1	Subpool/PCA	21/30	16	6	7	3
Syntax Set 1	Subpool/PCA	10/10	10	0	5	5
IDT Set 1	1-step assembly/PCA	12/15	10	4	2	4
Syntax Set 1	1-step assembly/PCA	14/14	10	5	4	1
Syntax Set 2	1-step assembly/PCA	10/18	10	1	5	4
Syntax Set 3	1-step assembly/PCA	11/12	10	1	4	5

ANALYSIS OF COST/TIME/ACCURACY

Cost and Time Analysis

oligo source	IDT	Syntax
synthesis time/time to delivery	2 days	13 hrs
oligo # produced	32	96
[oligo] yield nmol	100	0.3
oligo length	90	60
cost/kit or order	$687	$2,470
cost/96 oligos	$2,062	$2,470
cost per oligo	$21	$26
cost per base	$0.24	$0.43
assembly time	1-2 days	1-2 days
% perfect assembled sequences	38% (n=26)	18% (n=40)

SUMMARY AND CONCLUSIONS

1. **We assessed the first to market enzymatic DNA synthesizer from DNAScript (Syntax-100), making oligos to assemble and clone the gene for GFP**. We compared our enzymatically produced oligos with chemically synthesized oligos (IDT).
2. The Syntax-100 was very easy to use with a 15-min setup time, 13hr synthesis and oligo-cleanup time, and a self-contained aqueous waste stream – in contrast to the large volumes of hazardous chemicals required for chemical DNA synthesis.
3. We were able to produce 2 sets of 96 oligonucleotides over 2 days, including washing/system maintenance, and oligos were quantitated and normalized on the plate, ready for use at the end each run.
4. The cost/oligo is comparable between IDT and Syntax, although the overall yield for the Syntax is much lower (300X), but sufficient for downstream applications as evidenced by PCR assembly results.
5. Chemical DNA synthesis is reported to have 4X more accuracy in overall synthesis compared to enzymatic synthesis, and in our experiments we saw ~2X more perfect sequences assembled using IDT vs Syntax oligos**.**
6. **Overall we were able to produce more oligos (#) in a shorter time span with comparable accuracy/cost with the Syntax compared to ordering from IDT** with control over final yield and sequence-editing capabilities, as well as possible modifications including biotin, fluorophore, quencher etc. for many potential downstream applications.

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