Supporting Information

# Parallel, Large Scale and Long Synthetic Oligodeoxynucleotide Purification Using the Catching Full-Length Sequence by Polymerization Technique

Dhananjani N. A. M. Eriyagama,<sup>†</sup> Shahien Shahsavari,<sup>†</sup> Bhaskar Halami,<sup>†</sup> Bao-Yuan Lu,<sup>\*,‡</sup> Fengping Wei<sup>§</sup> and Shiyue Fang<sup>\*,†</sup>

† Department of Chemistry, Michigan Technological University, 1400 Townsend Drive, Houghton, MI 49931, USA

‡ Nalco Champion, an Ecolab Company, 11177 S. Stadium Drive, Sugar Land, TX 77478, USA

§ CGeneTech, Inc., 7202 E. 87th Street, Suite#100, Indianapolis, IN 46256, USA

#### Table of contents

ODN sequences	.S2-S3
RP HPLC of ODNs	.S3-S20
MALDI-TOF MS of ODNs	S21-S28
Photo of ODN 1m	S28-S28
LC-MS data for ODN <b>1a-c</b>	.S29-S45
UV spectra and OD <sub>260</sub> of ODNs	.S46-S57

### Table S1. ODN sequences

ODN	length	sequence
1a	15-mer	TTT ATT CCC CAA CAT
1b	21-mer	CAA AGT AGC GTG CAC TTT TGC
1c	23-mer	TCA CAT GAT ACC ATT CTC CTA AT
1d	19-mer	TCG GCA TAC CAT GAT TAC T
1e	20-mer	TAC TTG CTG CTT AGA CCG CT
1f	20-mer	CCC ACG TTT TAG CGC TTC GT
1g	20-mer	CTG TGT CGC ATG TAA AAG GT
1h	21-mer	CAC GCT TCA TGA TAT AAC CCT
<b>1i</b>	21-mer	TTG CCA TGA TTG ACA ACC AAT
1j	22-mer	CAA TGG AAG TAC CAT TGA TAC T
1k	26-mer	TAG ACC TAC TAG ATA GGT TCC CAC GT
11	28-mer	TAA TGG AAG TAC CAT TGA TAC TAC CAT T
1m	32-mer	TAG TTT TAT AAT TTC ATC AGC AGT GTT ACC GT
1n	43-mer	TCG GCT CAA CTC AAA CCTATC AAA CTT GTA ACC CCT CGG CGC T
10	64-mer	TAT TAT AGC ATA CTC TGT AAT AGT TGT CAA ACA TTA ATA GTT AAT TCT CCC ATT CTA AAA CGA T
1р	80-mer	TAC TCA TAA TAC TGT TTA CCG TCA TCA TCT TGA AGC AAC ATT GTC ACA TCG TAT GAG TCA ACA AAA TCA TTT TGC ACC AT
1q	90-mer	TAT TAT AGC ATA CTC TGT AAT AGT TGT CAA ACA TTA ATA GTT AAT TCT CCC ATT CTA AAA CGA TTT GAT CGT TTA TTT CTA CAA TTA GAT
1r	110-mer	CAT TAT AGC ATA CTC TGT AAT AGT TGT CAA ACA TTA ATA GTT AAT TCT CCC ATT CTA AAA CGA TTT GAT CGT TTA TTT CTA CAA TTA GAT GGA CTC CAT TTG TAC CGA AT
1s	151-mer	TCA ACA AAA TCA TTT TGC ACC ATG TGG AGC ACC TCC AAA TAA CAC CTT TAT AAC CCA TGT GGC GTA ATC ATT GTT TTC CAT CCT AGA AAG CTC ATA CAA TGC GTT TTT CAT GAG TTT ATT TTC ATG CTC TAG TTT AGT CAT CTT CTT TTC T
1t	197-mer	CCC CAA CAT ACA CAT GAC AAT GGA AGT ACC GTA CCA TTG ATA CTA CCA TTA TAG CAT ACT CTG TAA TAG TTG TCA AAC ATT AAT

AGT TAA TTC TCC CAT TCT AAA ACG ATT TGA TCG TTT ATT TCT ACA ATT AGA TGG ACT CCA TTT GTA CCG AAT GGA TGG ACT TGT AAC TTT ATC GTA CCA TCT TTA AAC ATA TT

- 1u203-merTTT AAT CCC CAA CAT ACA CAT GAC AAT GGA AGT ACC GTA CCA<br/>TTG ATA CTA CCA TTA TAG CAT ACT CTG TAA TAG TTG TCA AAC<br/>ATT AAT AGT TAA TTC TCC CAT TCT AAA ACG ATT TGA TCG TTT<br/>ATT TCT ACA ATT AGA TGG ACT CCA TTT GTA CCG AAT GGA TGG<br/>ACT TGT AAC TTT ATC GTA CCA TCT TTA AAC ATA TT
- 1v225-merTTT AAT CCC CAA CAT ACA CAT GAC AAT GGA AGT ACC GTA CCA<br/>TTG ATA CTA CCA TTA TAG CAT ACT CTG TAA TAG TTG TCA AAC<br/>ATT AAT AGT TAA TTC TCC CAT TCT AAA ACG ATT TGA TCG TTT<br/>ATT TCT ACA ATT AGA TGG ACT CCA TTT GTA CCG AAT GGA TGG<br/>ACT TGT AAC TTT ATC GTA CCA TCT TTA AAC ATA TTC GCA ATA<br/>TGT TCT TCT AAC TCT
- 1w251-merTTT AAT CCC CAA CAT ACA CAT GAC AAT GGA AGT ACC GTA CCA<br/>TTG ATA CTA CCA TTA TAG CAT ACT CTG TAA TAG TTG TCA AAC<br/>ATT AAT AGT TAA TTC TCC CAT TCT AAA ACG ATT TGA TCG TTT<br/>ATT TCT ACA ATT AGA TGG ACT CCA TTT GTA CCG AAT GGA TGG<br/>ACT TGT AAC TTT ATC GTA CCA TCT TTA AAC ATA TTC GCA ATA<br/>TGT TCT TCT AAC TCT GCA CGC TTC ATG ATA TAA CCC TCC TT
- 1x275-merTTT AAT CCC CAA CAT ACA CAT GAC AAT GGA AGT ACC GTA CCA<br/>TTG ATA CTA CCA TTA TAG CAT ACT CTG TAA TAG TTG TCA AAC<br/>ATT AAT AGT TAA TTC TCC CAT TCT AAA ACG ATT TGA TCG TTT<br/>ATT TCT ACA ATT AGA TGG ACT CCA TTT GTA CCG AAT GGA TGG<br/>ACT TGT AAC TTT ATC GTA CCA TCT TTA AAC ATA TTC GCA ATA<br/>TGT TCT TCT AAC TCT GCA CGC TTC ATG ATA TAA CCC TCC TTA<br/>TCA GAT GTC AAA TAT AGT TTT CT
- 1y303-merTTT AAT CCC CAA CAT ACA CAT GAC AAT GGA AGT ACC GTA CCA<br/>TTG ATA CTA CCA TTA TAG CAT ACT CTG TAA TAG TTG TCA AAC<br/>ATT AAT AGT TAA TTC TCC CAT TCT AAA ACG ATT TGA TCG TTT<br/>ATT TCT ACA ATT AGA TGG ACT CCA TTT GTA CCG AAT GGA TGG<br/>ACT TGT AAC TTT ATC GTA CCA TCT TTA AAC ATA TTC GCA ATA<br/>TGT TCT TCT AAC TCT GCA CGC TTC ATG ATA TAA CCC TCC TTA<br/>TCA GAT GTC AAA TAT AGT TTT CTC ACG GCT CAA CTC AAA CCT<br/>ATC AAA CTT

































































S21



S22





S24







m/z



S27



Photo of ODN 1m from large scale purification using the catching full-length sequence by polymerization technology



The following pages (S29-S34) contain LC-MS data for ODN 1a

Column	Agilent Extended C-18, 1.8 μm, 50 × 2.1 mm
Mobile phase	A: 200 mM HFIP, 8.1 mM TEA in water
	B: Methanol
Gradient	Time: 0-1-11-11.5-14.5-15 (min)
	B%: 10-10-70-90-90-10
	Post run: 10 min
Flow rate: (mL/min)	0.2
Column temp	40 °C
Injection Volume	2 uL
Detection	Agilent 1200 Series HPLC with Agilent 6224 Time-of- Flight LC/MS Electrospray Ionization (ESI), Negative Mode, 400-3200 mAu, data collection started at 2 min after injection.













## The following pages (S35-S39) contain LC-MS data for ODN 1b

Column	Agilent Extended C-18, 1.8 µm, 50 × 2.1 mm
Mobile phase	A: 200 mM HFIP, 8.1 mM TEA in water
	B: Methanol
Gradient	Time: 0-1-11-11.5-14.5-15 (min)
	B%: 10-10-70-90-90-10
	Post run: 10 min
Flow rate: (mL/min)	0.2
Column temp	40 °C
Injection Volume	2 uL
Detection	Agilent 1200 Series HPLC with Agilent 6224 Time-of- Flight LC/MS Electrospray Ionization (ESI), Negative Mode, 400-3200 mAu, data collection started at 2 min after injection.











# The following pages (S40-S45) contain LC-MS data for ODN 1c

Column	Agilent Extended C-18, 1.8 μm, 50 × 2.1 mm
Mobile phase	A: 200 mM HFIP, 8.1 mM TEA in water
	B: Methanol
Gradient	Time: 0-1-11-11.5-14.5-15 (min)
	B%: 10-10-70-90-90-10
	Post run: 10 min
Flow rate: (mL/min)	0.2
Column temp	40 °C
Injection Volume	2 uL
Detection	Agilent 1200 Series HPLC with Agilent 6224 Time-of- Flight LC/MS Electrospray Ionization (ESI), Negative Mode, 400-3200 mAu, data collection started at 2 min after injection.











## Other small elution peaks: same ions as in solvent blank







UV of 15-mer ODN **1a**. The ODN was synthesized on a 1 µmol scale. One fifth of the CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 100 µL of water, and 10 µL was taken out and diluted to 2 mL to obtain the UV spectrum. The OD<sub>260</sub> of the 1 µmol synthesis and purification was calculated to be 28.84 (0.2884 × 5 × 100 µL ÷ 10 µL × 2 mL ÷ 1 mL).



UV of 21-mer ODN **1b**. The ODN was synthesized on a 1 µmol scale. One fifth of the CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 100 µL of water, and 10 µL was taken out and diluted to 2 mL to obtain the UV spectrum. The OD<sub>260</sub> of the 1 µmol synthesis and purification was calculated to be 31.98 (0.3198 × 5 × 100 µL ÷10 µL × 2 mL ÷ 1 mL).



UV of 23-mer ODN **1c**. The ODN was synthesized on a 1 µmol scale. One fifth of the CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 100 µL of water, and 10 µL was taken out and diluted to 2 mL to obtain the UV spectrum. The OD<sub>260</sub> of the 1 µmol synthesis and purification was calculated to be 26.64 (0.2664 × 5 × 100 µL ÷ 10 µL × 2 mL ÷ 1 mL).



UV of 19-mer ODN **1d**. The ODN was synthesized on a 1 µmol scale. One fifth of the CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 100 µL of water, and 10 µL was taken out and diluted to 2 mL to obtain the UV spectrum. The OD<sub>260</sub> of the 1 µmol synthesis and purification was calculated to be 74.4 (0.744 × 5 × 100 µL ÷ 10 µL × 2 mL ÷ 1 mL).



UV of 20-mer ODN **1e**. The ODN was synthesized on a 1 µmol scale. One fifth of the CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 100 µL of water, and 10 µL was taken out and diluted to 2 mL to obtain the UV spectrum. The OD<sub>260</sub> of the 1 µmol synthesis and purification was calculated to be 68.7 (0.687 × 5 × 100 µL ÷ 10 µL × 2 mL ÷ 1 mL).



UV of 20-mer ODN **1f**. The ODN was synthesized on a 1 µmol scale. One fifth of the CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 100 µL of water, and 10 µL was taken out and diluted to 2 mL to obtain the UV spectrum. The OD<sub>260</sub> of the 1 µmol synthesis and purification was calculated to be 11.75 (0.1175 × 5 × 100 µL ÷ 10 µL × 2 mL ÷ 1 mL).



UV of 20-mer ODN **1g**. The ODN was synthesized on a 1 µmol scale. One fifth of the CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 100 µL of water, and 10 µL was taken out and diluted to 2 mL to obtain the UV spectrum. The OD<sub>260</sub> of the 1 µmol synthesis and purification was calculated to be 21.96 (0.2196 × 5 × 100 µL ÷ 10 µL × 2 mL ÷ 1 mL).



UV of 20-mer ODN **1h**. The ODN was synthesized on a 1 µmol scale. One fifth of the CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 100 µL of water, and 10 µL was taken out and diluted to 2 mL to obtain the UV spectrum. The OD<sub>260</sub> of the 1 µmol synthesis and purification was calculated to be 28.42 (0.2842 × 5 × 100 µL ÷ 10 µL × 2 mL ÷ 1 mL).



UV of 22-mer ODN **1**j. The ODN was synthesized on a 1 µmol scale. One fifth of the CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 100 µL of water, and 10 µL was taken out and diluted to 2 mL to obtain the UV spectrum. The OD<sub>260</sub> of the 1 µmol synthesis and purification was calculated to be 21.73 (0.2173 × 5 × 100 µL ÷ 10 µL × 2 mL ÷ 1 mL).



UV of 26-mer ODN **1k**. The ODN was synthesized on a 1 µmol scale. One fifth of the CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 100 µL of water, and 10 µL was taken out and diluted to 2 mL to obtain the UV spectrum. The OD<sub>260</sub> of the 1 µmol synthesis and purification was calculated to be 99.6 (0.996 × 5 × 100 µL ÷ 10 µL × 2 mL ÷ 1 mL).



UV of 28-mer ODN **1I**. The ODN was synthesized on a 1 µmol scale. One fifth of the CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 100 µL of water, and 10 µL was taken out and diluted to 2 mL to obtain the UV spectrum. The OD<sub>260</sub> of the 1 µmol synthesis and purification was calculated to be 15.74 (0.1574 × 5 × 100 µL ÷ 10 µL × 2 mL ÷ 1 mL).



UV of 32-mer ODN **1m**. The ODN was synthesized on a 60 µmol scale. All CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 5 mL of water, and 5 µL was taken out and diluted to 40 mL to obtain the UV spectrum. The OD<sub>260</sub> of the 60 µmol synthesis and purification was calculated to be 13,076 (0.3269 × 5 mL ÷ 5 µL × 40 mL ÷ 1 mL).



UV of 43-mer ODN **1n**. The ODN was synthesized on a 0.2 µmol scale. All CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 200 µL of water, and 20 µL was taken out and diluted to 2 mL to obtain the UV spectrum. The  $OD_{260}$  of the 0.2 µmol synthesis and purification was calculated to be 9.664 (0.4832 × 200 µL ÷ 20 µL × 2 mL ÷ 1 mL).



UV of 64-mer ODN **1o**. The ODN was synthesized on a 0.2 µmol scale. All CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 200 µL of water, and 20 µL was taken out and diluted to 2 mL to obtain the UV spectrum. The  $OD_{260}$  of the 0.2 µmol synthesis and purification was calculated to be 4.106 (0.2053 × 200 µL ÷ 20 µL × 2 mL ÷ 1 mL).



UV of 80-mer ODN **1p**. The ODN was synthesized on a 0.2 µmol scale. All CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 200 µL of water, and 20 µL was taken out and diluted to 2 mL to obtain the UV spectrum. The OD<sub>260</sub> of the 0.2 µmol synthesis and purification was calculated to be 15.04 (0.7520 × 200 µL ÷ 20 µL × 2 mL ÷ 1 mL).



UV of 90-mer ODN **1q**. The ODN was synthesized on a 0.2 µmol scale. All CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 200 µL of water, and 20 µL was taken out and diluted to 2 mL to obtain the UV spectrum. The OD<sub>260</sub> of the 0.2 µmol synthesis and purification was calculated to be12.746 (0.6373 × 200 µL ÷ 20 µL × 2 mL ÷ 1 mL).



UV of 110-mer ODN **1r**. The ODN was synthesized on a 0.2 µmol scale. All CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 200 µL of water, and 20 µL was taken out and diluted to 2 mL to obtain the UV spectrum. The OD<sub>260</sub> of the 0.2 µmol synthesis and purification was calculated to be 8.074 (0.4037 × 200 µL ÷ 20 µL × 2 mL ÷ 1 mL).



UV of 151-mer ODN **1s**. The ODN was synthesized on a 0.2 µmol scale. All CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 200 µL of water, and 20 µL was taken out and diluted to 2 mL to obtain the UV spectrum. The  $OD_{260}$  of the 0.2 µmol synthesis and purification was calculated to be 2.61(0.1305 × 200 µL ÷ 20 µL × 2 mL ÷ 1 mL).



UV of 197-mer ODN **1t**. The ODN was synthesized on a 0.2 µmol scale. All CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 200 µL of water, and 20 µL was taken out and diluted to 2 mL to obtain the UV spectrum. The OD<sub>260</sub> of the 0.2 µmol synthesis and purification was calculated to be 2.848 (0.1424 × 200 µL ÷ 20 µL × 2 mL ÷ 1 mL).



UV of 203-mer ODN **1u**. The ODN was synthesized on a 0.2 µmol scale. All CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 200 µL of water, and 20 µL was taken out and diluted to 2 mL to obtain the UV spectrum. The  $OD_{260}$  of the 0.2 µmol synthesis and purification was calculated to be 9.3 (0.465 × 200 µL ÷ 20 µL × 2 mL ÷ 1 mL).



UV of 225-mer ODN **1v**. The ODN was synthesized on a 0.2 µmol scale. All CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 200 µL of water, and 20 µL was taken out and diluted to 2 mL to obtain the UV spectrum. The  $OD_{260}$  of the 0.2 µmol synthesis and purification was calculated to be 6.688 (0.3344 × 200 µL ÷ 20 µL × 2 mL ÷ 1 mL).



UV of 251-mer ODN **1w**. The ODN was synthesized on a 0.2 µmol scale. All CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 200 µL of water, and 20 µL was taken out and diluted to 2 mL to obtain the UV spectrum. The OD<sub>260</sub> of the 0.2 µmol synthesis and purification was calculated to be 7.316 (0.3658 × 200 µL  $\div$  20 µL × 2 mL  $\div$  1 mL).



UV of 275-mer ODN **1x**. The ODN was synthesized on a 0.2 µmol scale. All CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 200 µL of water, and 20 µL was taken out and diluted to 2 mL to obtain the UV spectrum. The  $OD_{260}$  of the 0.2 µmol synthesis and purification was calculated to be 5.456 (0.2728 × 200 µL ÷ 20 µL × 2 mL ÷ 1 mL).



UV of 303-mer ODN **1y**. The ODN was synthesized on a 0.2 µmol scale. All CPG was subjected to deprotection and cleavage. The purified ODN was dissolved in 200 µL of water, and 20 µL was taken out and diluted to 2 mL to obtain the UV spectrum. The OD<sub>260</sub> of the 0.2 µmol synthesis and purification was calculated to be 13.012 (0.6506 × 200 µL ÷ 20 µL × 2 mL ÷ 1 mL).