

# INTEGRATED DNA TECHNOLOGIES PRAJMERI I PROBE ZA qPCR





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# DNA OLIGOS AND ULTRAMER DNA OLIGOS

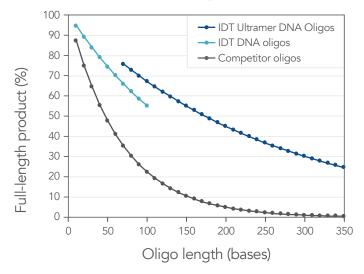
Generate consistently reliable data from the highest fidelity oligos available

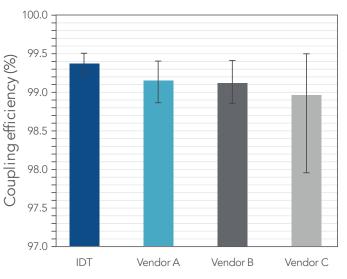


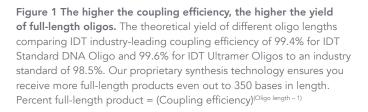
All single-stranded and duplexed sequences are produced with industry leading coupling efficiencies, resulting in higher quality DNA products. Our proprietary technologies allow us to produce high quality Ultramer<sup>™</sup> DNA Oligos, long oligos up to 200 bases. (Figures 1–2).

To push the limits of oligo synthesis, we developed specialized platforms that allow us to deliver the highest quality PCR primers, dual-labelled probes for qPCR, indexed adapters for NGS, long biotinylated oligos for NGS target capture, and other advanced and custom products.

Each oligo undergoes extensive quality analysis, including evaluation by ESI-mass spectrometry to ensure sequence composition\*. Our manufacturing processes are standardized at every production site around the world, so you consistently receive the highest quality oligos.







**Figure 2. Consistently high coupling efficiency from IDT synthesis.** Unmodified 30–45 base oligos (n = 64) were ordered from different suppliers over a 12-month period. IDT oligos had the highest coupling efficiency, and exhibited the smallest variance around the mean.

\* With the exception of mixed base oligos, which could potentially represent multiple sequences and therefore, cannot be accurately evaluated by ESI mass spectrometry.

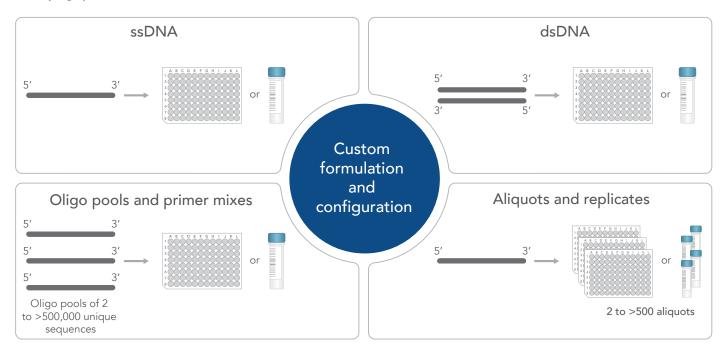
#### > WWW.IDTDNA.COM

#### MODIFICATIONS AND MODIFIED BASES

Select from more than 400 modifications including: quenchers, spacers, linkers, modified bases, fluorophores, and modifications for click chemistry. Our technical support team can provide guidance on which modifications are best suited to your specific application. Learn more at www.idtdna.com/mods.

#### CUSTOM FORMULATION AND PACKAGING

Customized services from primer mixes and oligo duplexes, to pools of tens of thousands of unique oligos in equal or varying quantities are available. Learn more at www.idtdna.com/formulations.



#### SCITOOLS<sup>™</sup> WEB TOOLS

Plan your experiments and design oligos that perform optimally for your conditions with our online software tools. The OligoAnalyzer<sup>M</sup> and UNAFold tools allow you to determine GC content, sequence complement, and secondary structure characteristics such as melting temp ( $T_m$ ) and self-complementarity. The PrimerQuest<sup>M</sup> Tool can be used to design primers and probes for PCR-based applications. Learn more about these tools, and additional applications at www.idtdna.com/SciTools.

For standard, desalted oligos ≥20 bases, we offer the following yield guarantees:

Product	Available length	Guaranteed yield*	
25 nmol DNA oligo	15–60 bases	10 nmol	
100 nmol DNA oligo	10-90 bases	30 nmol	
250 nmol DNA oligo	5–100 bases	50 nmol	

#### > FOR MORE INFORMATION AND TO ORDER, VISIT WWW.IDTDNA.COM/DNA.

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# PRIMETIME<sup>™</sup> qPCR PROBES

Double- and single-quenched probes for use in 5' nuclease assays



### DYES AND QUENCHERS FOR EVERY EXPERIMENT

PrimeTime qPCR Probes provide reliable sensitivity even in demanding applications such as multiplexing and digital PCR. PrimeTime qPCR Probes are available in a wide variety of dye-quencher combinations (Table 1) that are compatible with common qPCR instruments.

#### ACHIEVE CONSISTENT RESULTS

All PrimeTime Probes are HPLC purified, and then verified by mass spectrometry, to deliver batch-to-batch consistency and minimize the need for troubleshooting.

#### Table 1. Commonly used fluorophores and quenchers

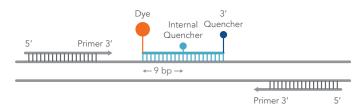
Fluorophore	Emission wavelength (nm)	Quencher		
6-FAM*	520			
SUN™*	554			
JOE™*	555	ZEN/Iowa Black™ FQ		
HEX*	555			
MAX <sup>™</sup> *	557			
Cy <sup>®</sup> 3	564			
ATTO <sup>™</sup> 550§	575			
ROX	608			
Texas Red <sup>®</sup> -X	617	Iowa Black RQ <sup>†‡</sup>		
ATTO 647N§	662			
Cy 5 <sup>¥</sup>	668			
Су 5.5	706	Black Hole Quencher®-31		

- \* Probes with 6-FAM, SUN, JOE, MAX, or HEX fluorophores are also available as traditional, single-quenched probes with either Iowa Black FQ (license free) or Black Hole Quencher-1 (additional third-party licenses required for diagnostic use).
- + Black Hole Quencher-2 (BHQ2) may also be used as a quencher (additional thirdparty licenses required for diagnostic use).
- ‡ Double-quenched probes available as a custom order.
- § ATTO-labeled probes available as a custom order.
- ¥ Cy 5 is also available as a single-quenched probe with BHQ2 (additional third-party licenses required for diagnostic use).
- $\P$  Available as research use only.

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### IMPROVE ASSAY SENSITIVITY WITH DOUBLE-QUENCHED PROBES

Reduce background and increase assay sensitivity with ZEN or TAO Double-Quenched Probes. Our exclusive internal quenchers are 9 bases from the 5' fluorophore and work in combination with the 3' Iowa Black quencher for maximum probe performance (Figure 1).



**Figure 1.** Schematic of a PrimeTime qPCR 5' Nuclease Assay using a double-quenched probe that includes a dye, a ZEN or TAO internal quencher, and a 3' quencher.

With nearly 4 times lower background fluorescence (Figure 2A) and approximately 30% increased signal (Figure 2B), ZEN Double-Quenched Probes simply perform better. See performance data for TAO Double-Quenched Probes at **www.idtdna.com/qPCRprobes**.

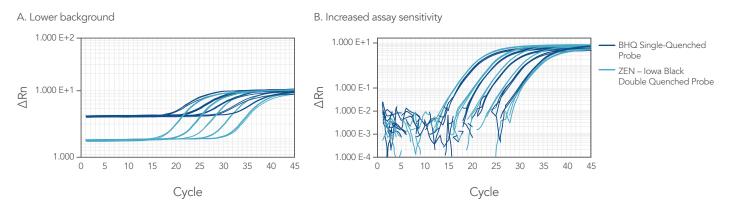
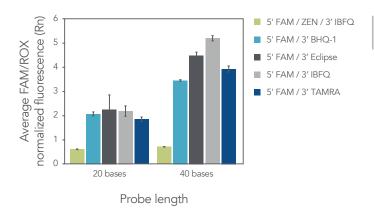


Figure 2. Increased signal detection and assay sensitivity from ZEN Double-Quenched Probes. (A) ZEN Probes (light blue) provide greater dye quenching, producing lower background and, therefore, higher signal intensities than standard single-quenched probes (BHQ Probes; dark blue). (B) ZEN Probes increase assay sensitivity, as demonstrated by the earlier Cq values observed compared to standard, BHQ single-quenched probes.

### ACHIEVE MAXIMUM QUENCHING FOR LONG PROBES

Effective quenching for ZEN Double-Quenched Probes as long as 40 bases means more effective designs, even for AT-rich targets.



**Figure 3. Only ZEN Double-Quenched Probes maintain low background signal with increasing probe length.** Probes of 2 lengths (20 or 40 bases) with 5 different quenchers were compared in 10 singleplex qPCRs. Six replicate reactions with each probe type were run with 50 ng of cDNA and the TaqMan<sup>®</sup> Gene Expression Master Mix (Thermo Fisher) under standard cycling conditions on the Applied Biosystems 7900HT system. Key: IBFQ = Iowa Black FQ Quencher (IDT); BHQ-1 = Black Hole Quencher-1 (Biosearch Technologies); MGB Eclipse<sup>®</sup> = Eclipse quencher (ELITech Group).

### ORDERING INFORMATION

Visit www.idtdna.com/qPCRprobes to enter your sequence and choose modifications

#### > FOR MORE INFORMATION, VISIT WWW.IDTDNA.COM/qPCRPROBES

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	Max emission wavelength (nm) and dye											
	520	554	555	555	557	564	575	608	617	662	668	706
Instrument	6-FAM	SUN™	JOE™	HEX	МАХтм	Cy® 3	ATTO™	ROX	Texas	ATTO	Cy 5	Cy 5.5
							550#		Red®	647N§		
Agilent		-	-									
Aria Mx	•	•			•	•	X	•			•	x
<b>Applied Biosystems</b>												
7500 (PRISM®)	•	•	•	•1	•	•	٠	#	•*	•	•	x
7500 HT (Fast)	٠	•	•	•1	•	•	•	#	•*	•	•	x
7700	٠	•	•	•	•	×	•	#	x	x	X	x
7900 (PRISM)	٠	•		-1	•	x	x	#	x	x	x	×
7900 HT	٠	•	•	-1	•	x	x	#	х	X	x	x
StepOne®	٢	•	•	-1		x	х	#	х	X	х	X
StepOne Plus®	•		•	•1	•	х	•	#	x	х	x	x
Viia 7	٠	<b>O</b>	•	•	•	•	•	#	•*	•		x
QuantStudio® 3	٠	•	•	•	•	٠	•	#	•*	x	х	x
QuantStudio 5	•	•	•	•	•	•	•	#	•*	•	•	•
QuantStudio 6 Flex	•	•	•	•1	•	•	•	#	•*	•	10	•
QuantStudio 7	•	•	•	-1	•	•	•	#	•*	•	•	•
QuantStudio 12k flex	•	•	•	•	•	•	•	#	•*	•	•	0
QuantStudio 3D	•	•	•		х	х	x	•*	х	х	х	x
Bio-Rad												
CFX384®	٠	•	•	•	•	x	х	•	•	•	•	х
CFX96®	0	•	•	0	•	х	x	•	•	•	•	•
Connect®	•	•	•	•1	х	х	х	х	х	x	х	x
MyIQ2	•	•		•	•	х	х	x	х	x	х	x
MyIQ5	•	•	•	•		•	0	•	•	•	•	х
QX100	•	•		_1	x	х	X	x	x	х	х	x
QX200	٠	•		•1	x	х	x	x	x	x	x	x
QX One <sup>™</sup>	٠	•	•	_1	x	x	x	x	х	•	- • -	•
Cepheid												
Smart iCycler®	•	•	•	•	•	•	x	•	•	•	0	x
Smart iCycler II	•	•	•			•	x	•	•	•	•	x
Illumina												
Eco	•	•	•	10	х	х	х	•	•	•	•	х
QIAGEN												
Rotor-gene® Q	•	•	•	0	•	х	х	•	•	•	•	•
Rotor-gene 6000	٠	•	•	0	•	х	x	•	0	•	•	x
Roche												
LightCycler® (LC) 2.0	•2	•	•2	•2	•2	•2	•2	•2	•2	•2	•2	•2
LC® 480	•	•	•	-01	• <sup>2</sup>	x	х	•2	<u>2</u>	•2	•2	х
LC 1536	٥	•	<u>2</u>	0	-2	х	х	x	х	x	х	x
LC Nano	•	•	<b>2</b>		<u>2</u>	•2	•2	•2	•2	•2	•2	•2
LC 96	•	•	•1	•1	2	x	x	•2	•2	•	•	x
Stilla		_		_								
Naica™	•	•	•	•	•	•	•	•	x	•	•	•
Combinati												
Absolute Q <sup>™</sup>	•	•	•	•		•	•	#	#	•	•	x
Fluidigm												
Biomark	•	•	•	•	•	•	x	٠	•	x	x	x
Dropworks												

# Dropworks Continuum<sup>™</sup>

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Supplier provided or recommended reporter dyes
Instrument capable dyes, but may require calibration
Instrument incapable of supporting
Instrument uses channel for a reference dye
Instrument works with VIC<sup>®</sup> (Life Technologies), so JOE or SUN can serve as an alternative if calibrated/tested
Roche recommends running color compensation for any dye set used
Cannot be used if ROX is used as a passive reference dye
Preferred dye equivalent for TAMRA
Preferred dye equivalent for Cy 5