Probes and custom assays for digital PCR (dPCR)

Cost-effective and conveniently packaged reagents for genotyping and gene expression applications using dPCR

Convenient packaging—probes provided in 0.5–2.5 nmol amounts

Fast turnaround time—start your experiments sooner

Cost effective—stretch your budget

Digital PCR (dPCR) is a quantitative PCR method that provides absolute quantification of DNA or RNA present in a sample [1]. It utilizes similar assay reagents as in standard qPCR measurements, but instead of analyzing the whole reaction, dPCR separates the mixture into individual nanoliter reactions (**Figure 1**). The final analysis counts the total number of individual partitions containing target molecules, providing an absolute measurement of the expression for the gene of interest. Digital PCR enables absolute quantification of molecules and can be especially useful when sample availability is limited. dPCR is amenable to many applications, including rare allele identification, liquid biopsy analysis, viral load quantification, single-cell analyses, and DNA quality control for sequencing [2].

IDT offers a variety of probes and custom assays for genotyping and gene expression applications using microwell or droplet-based dPCR platforms.

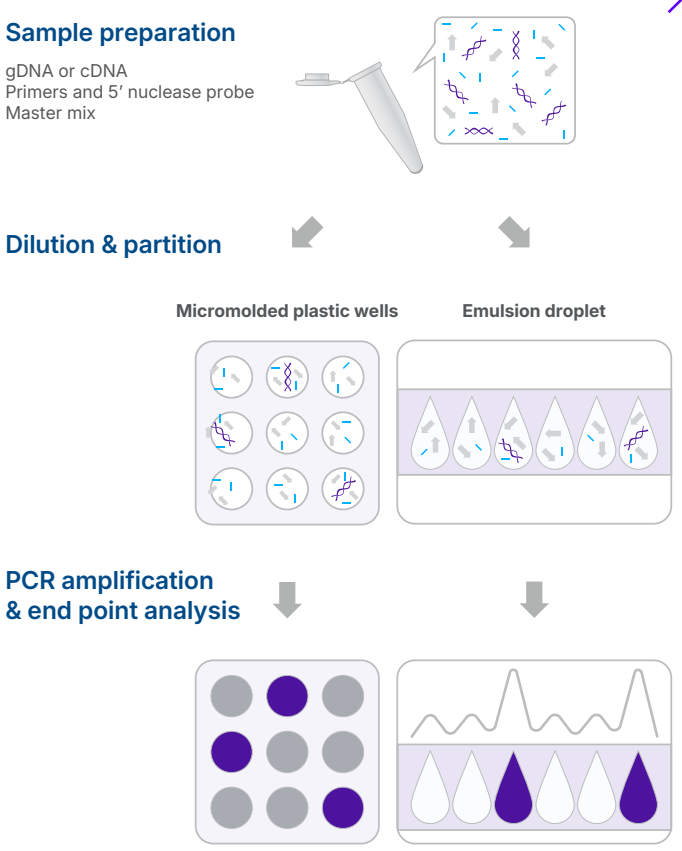

Figure 1. Digital PCR (dPCR) workflow. After isolation of genomic DNA (gDNA) or conversion of an mRNA sample to cDNA, master mix, target primers, and 5′ nuclease probe(s) are added. The mix is diluted and partitioned so there is either 1 or 0 targets in each partition. Some dPCR platforms partition into micromolded plastic wells; whereas, other dPCR platforms create emulsions of oil and water droplets. After PCR amplification, the fluorescent signal for each well or droplet is recorded. The number of positive wells or droplets provides an accurate count of the number of starting target gDNA or cDNA in the sample.

SNP genotyping applications

Use Affinity Plus™ qPCR Probes for enhanced discrimination of thermodynamically similar samples to identify single nucleotide polymorphisms and transcript variants. When incorporated into a probe, locked nucleic acids impart heightened structural stability [**3**], allowing for the use of shorter sequences with high melting temperatures (T_m). The Affinity Plus qPCR probes include up to 6 Affinity Plus monomers allowing flexible T_m adjustments (**Table 1**). Mini Affinity Plus qPCR Probes are ideal for dPCR applications, screening small sample sets.

Table 1. Mini Affinity Plus qPCR Probes.

5' reporter dye(s)	Emission (nm)	Quencher(s)	Delivery amount
FAM	520	Iowa Black™ FQ	0.5 nmol
SUN™	554		
HEX	555		
YAK	551		
Cy® 5	668	Iowa Black RQ	

For more information, to view larger delivery amounts (8 and 20 nmol minimum guarantee), or to place an order, go to **www.idtdna.com/AffinityProbes**.

For information on designing or ordering probes labeled with ATTO or other fluorophores not listed, contact us at **www.idtdna.com/ContactUs**.

Gene expression applications

PrimeTime™ qPCR Probes provide reliable, high-quality gene expression data. These 5′ nuclease probes are available with an assortment of reporter-dye combinations compatible with common qPCR instruments. ZEN™ and TAO™ Double-Quenched Probes from IDT reduce background fluorescence for longer probe designs. Reduced background improves the precision of dPCR applications. Choose from a variety of synthesis scales, dyes, and shipping options to meet your needs. Estimated turnaround times depend on the degree of probe complexity (**Table 2**). The Mini and Eco sizes are ideal for screening small sample sets or performing just a few reactions when optimizing probe designs. Larger scales are also available with customizable ratios of probes and primers.

Table 2. Primetime qPCR Probes—small scale synthesis.

5′ reporter dye	Emission (nm)	Quencher(s)	Delivery amount		Minimum guarantee
			Mini (0.5 nmol)	Eco (2.5 nmol)	Other scales (nmol)
FAM	520	ZEN/Iowa Black FQ	✓	✓	15, 25, 50
SUN	554	ZEN/Iowa Black FQ	✓	✓	10, 25, 50
HEX	555	ZEN/Iowa Black FQ	✓	✓	10, 25, 50
Cy 5	668	TAO/Iowa Black RQ	✓	✓	2, 8, 20

For more information, to view additional yield guarantee sizes, or to place an order, go to **www.idtdna.com/qPCRprobes**.

PrimeTime qPCR Probe Assays (Mini). This unit size is used for dPCR, screening small sample sets, or performing just a few reactions when optimizing probe designs. These custom assays consist of a primer pair and 5′ nuclease probe, and are available in various sizes, premixed, and shipped dried down in either tubes or plates (**Tables 3** and **4**). The probes/primers are supplied in the following ratio: 0.5/1.0 nmol. Larger unit sizes are available.

Table 3. PrimeTime Probe Assays in tubes.

5′ reporter dye	Emission (nm)	Quencher(s)	Unit size	Reactions	Other reaction sizes
FAM	520	ZEN/Iowa Black FQ	Mini	100	500, 2500
SUN	554	ZEN/Iowa Black FQ			
HEX	555	ZEN/Iowa Black FQ			
Cy 5	668	TAO/Iowa Black RQ			

Table 4. PrimeTime Probe Assays in 96-well plates.

5′ reporter dye	Emission (nm)	Quencher(s)	Unit size	Reactions	Other reaction sizes
FAM	520	ZEN/Iowa Black FQ	Mini	100	500, 2500

For more information, to view larger unit sizes, or to place an order, go to **www.idtdna.com/primetime-probe-assays**.

Table 5. Digital PCR instrument compatibility and reporter dyes.

	Max emission wavelength (nm) and dye											
	520	554	555	555	557	564	575	608	617	662	668	706
Instrument	6-FAM	SUN™	JOE™	HEX	MAX™	Cy® 3	ATTO™ 550#	ROX	Texas Red®-X	ATTO 647N§	Cy 5	Cy 5.5
Applied Biosystems												
QuantStudio® 3D	•	•	•	•	x	x	x	•*	x	x	x	x
Bio-Rad												
QX100™	•	•	•	•1	x	x	x	x	x	x	x	x
QX200™	•	•	•	•1	x	x	x	x	x	x	x	x
QX One™	•	•	•	•1	x	x	x	x	x	•	•	•
Stilla												
Naica™	•	•	•	•	•	•	•	•	x	•	•	•
Combinati												
Absolute Q™	•	•	•	•	•	•	•	#	#	•	•	x
Fluidigm												
Biomark	•	•	•	•	•	•	x	•	•	x	x	x
Dropworks												
Continuum™	•	•	•	•	x	x	x	•	x	•	•	x

•	Supplier provided or recommended reporter dyes
•	Instrument capable dyes, but may require calibration
x	Instrument incapable of supporting
#	Instrument uses channel for reference dye
*	Cannot be used if ROX is used as a passive reference dye
#	Preferred dye equivalent for TAMRA
§	Preferred dye equivalent for Cy 5

References

1. Taylor SC, Laperriere G, Germain H. **Droplet Digital PCR versus qPCR for gene expression analysis with low abundant targets: from variable nonsense to publication quality data**. *Sci Rep*. 2017;7(1):2409.
2. Quan PL, Sauzade M, Brouzes E. **dPCR: A Technology Review**. *Sensors* (Basel). 2018;18(4).
3. You Y, Moreira BG, Behlke MA, *et al*. **Design of LNA probes that improve mismatch discrimination**. *Nucleic Acids Res*. 2006;34(8):e60.

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