Transfer of commercial qPCR assays onto the QIAcuity® dPCR Platform

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Transfer of qPCR assays to dPCR

Digital PCR (dPCR) offers a number of advantages over quantitative PCR in various applications such as gene expression analysis (GEX), copy number variation (CNV) detection and single nucleotide polymorphism (SNP) analysis. Nonetheless, researchers may refrain from switching technologies, particularly those who have been using a well-established set of commercial qPCR assays for these applications.

Here, we demonstrate, how predesigned assays for GEX, CNV and SNP analyses, can be easily transferred from a qPCR platform to the QIAcuity dPCR System. Application-specific recommendations for setup and cycling protocols are given together with a side-by-side comparison of qPCR and dPCR results.

Experimental outline

- Test assays using qPCR with master mixes, instruments and cycling all as per manufacturers' recommendations.
- Test assays using qPCR with QIAcuity Probe PCR Master Mix (for dPCR) on identical qPCR instrument with cycling as recommended for dPCR.
- Test assays using dPCR with QIAcuity Probe PCR Master Mix on QIAcuity instrument with recommended dPCR cycling.

Assays tested and targets

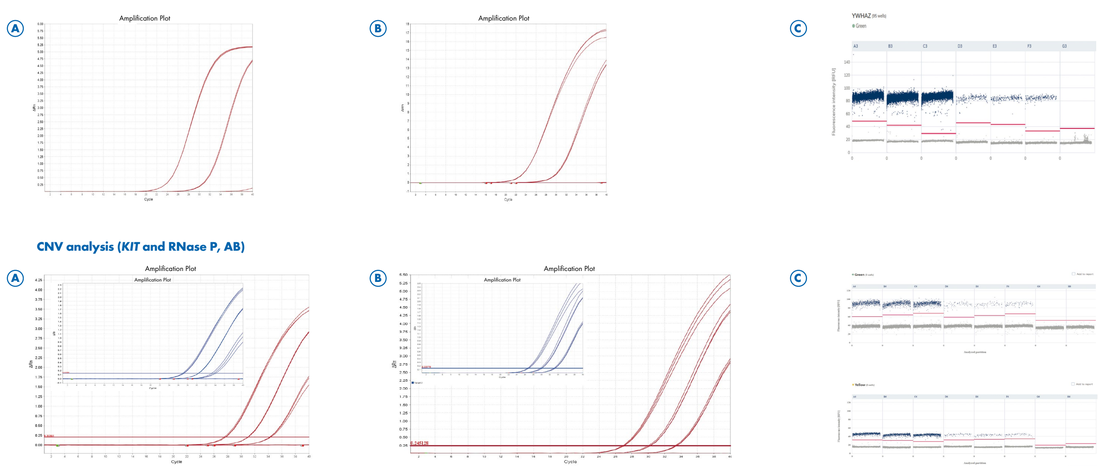
Assay type	GEX assays	CNV assays	SNP assays	
Supplier	AB, BR, IDT	AB	AB	
	Gene	Gene	dbSNP	Gene
Targets	*ERBB2*	*FLT1*	rs2272998	*SASH1*
	EGFR	*FLT3*	rs279844	*GABRA2*
	CDKN2A	*KDR*	rs214955	*SYNE1*
	CDK1	*KIT*	rs2503107	*RSPO3*
	KDR	*FLT4*	rs13134862	*RCHY1*
	HPRT1	*EGFR*	rs1410059	*SORBS1*
	RPL13A	*RRM1*	rs13218440	*HIVEP1*
	PPIA	*TYMS*	rs985492	*B4GALT6*
	B2M	*BRAF*	rs740598	*HSPA12A*
	EEF2	*TOP2A*	rs1058083	*UBAC2*
	TUBA4A	*VEGFA*	rs16942	*BRCA1*
	UBC	*FGFR1*	rs799917	*BRCA1*
	YWHAZ	*MYC*	rs144848	*BRCA2*
	HMBS	*MET*	rs1799944	*BRCA2*
	SDHA	*MDM2*	rs1128503	*ABCB1/MDR*
	PCBP1	*PIK3CA*	rs2032582	*ABCB1/MDR*
	TOP1	*TFDP1*	rs1045642	*ABCB1/MDR*
	RPS18	*PDGFRA*	rs1006737	*CACNA1C*
	UBE2D2	*SRY/SOX2*	rs755622	*MIF*
	HDAC3	*IGF1R*	rs12143842	*NOS1AP*

AB: Applied Biosystems; BR: Bio-Rad; IDT:Integrated DNA Technologies

Exemplary results obtained in qPCR and dPCR: GEX and CNV

Out of 80 qPCR assays tested, 79 assays were easily transferred to qPCR on the QIAcuity System. One assay failed in dPCR and was also non-functional with the manufacturer's master mix. For CNV assays, minor adjustments of the primer/probe concentration were implemented (C).

GEX analysis (*YWHAZ*, IDT)



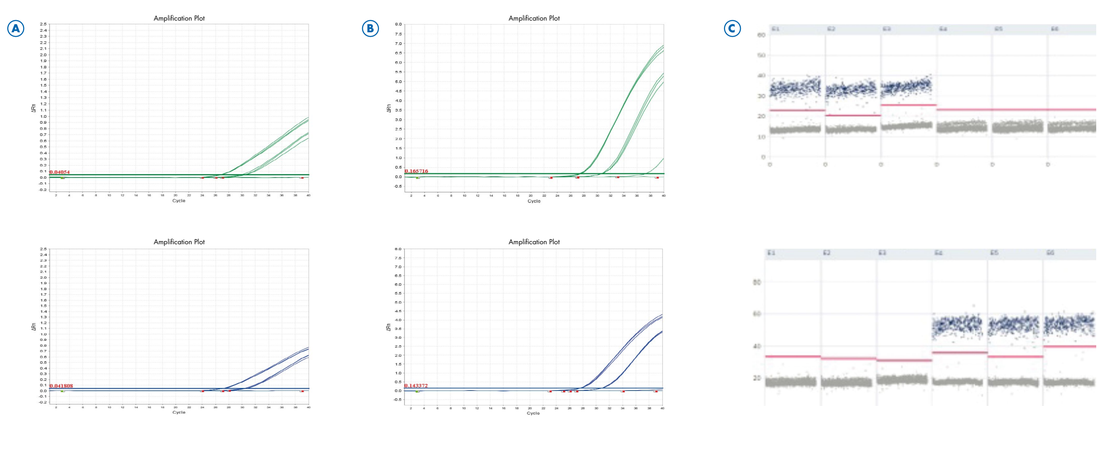
CNV analysis (*KIT* and RNase P, AB)

GEX and CNV analysis. qPCR assays shown were tested **A** using qPCR and assay suppliers' master mixes, **B** using qPCR and QIAcuity Probe PCR Mastermix and **C** using dPCR on the QIAcuity Four System with QIAcuity Probe PCR Mastermix. Insert: Reference target RNase P.

Exemplary results obtained in qPCR and dPCR: SNP

All 20 qPCR assays tested were easily transferred to qPCR on the QIAcuity System. For SNP assays, minor adjustments of the cycling protocol were implemented (C).

SNP analysis (rs214955, SYNE1)

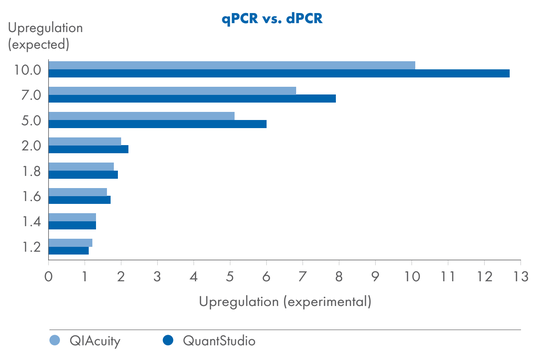


SNP analysis. qPCR assays shown were tested **A** using qPCR and assay suppliers' master mixes, **B** using qPCR and QIAcuity Probe PCR Mastermix and **C** using dPCR on the QIAcuity Four System with QIAcuity Probe PCR Mastermix. Upper panels: SNP1 (FAM™); lower panels: SNP2 (VIC™).

Comparison of qPCR assays for gene expression analysis in qPCR and dPCR

Pairwise comparison, using identical templates, shows higher accuracy of GEX analysis in dPCR.

Upregulation expected: *UBE2D2*	QuantStudio	QIAcuity
1.2	1.1	1.2
1.4	1.3	1.3
1.6	1.7	1.6
1.8	1.9	1.8
2.0	2.2	2.0
5.0	6.0	5.1
7.0	7.9	6.8
10.0	12.7	10.1



Experimental setup

Template: cDNA (obtained from Universal Human Reference RNA, spiked with increasing number of copies for target of interest (synthetic template), up to tenfold

Assays: PrimeTime™ assays (IDT) *UBE2D2* (Ubiquitin conjugating enzyme, target of interest, FAM™), and *YWHAZ* (reference target, HEX™), QIAcuity Probe PCR Master Mix and PrimeTime Gene Expression Master Mix (IDT)

Instruments: QIAcuity Four and QuantStudio® 5 (AB)

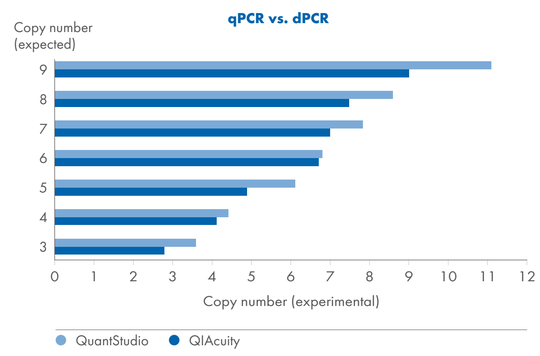
Cycling: According to manufacturers' recommendations

Analysis: QIAcuity Software Suite 2.0.20 and QuantStudio Copy Caller Software (AB)

Comparison of qPCR assays for CNV analysis in qPCR and dPCR

Pairwise comparison, using identical templates, shows higher accuracy of CNV analysis in dPCR.

Expected copy number	QIAcuity	QuantStudio 5
2	2	2
3	2.8	3.6
4	4.1	4.4
5	4.9	6.1
6	6.7	6.8
7	7.0	7.8
8	7.5	8.6
9	9.0	11.1



Experimental setup

Template: gDNA (2 copies/genome) spiked with increasing number of extra MET copies (synthetic template) up to 9 copies in total.

Assays: TaqMan® CNV assays (AB) for MET (target of interest, FAM, 1x), and RNase P (reference target, VIC, 0.25x for dPCR), QIAcuity Probe PCR Mastermix and TaqPath™ ProAmp™ Master Mix (AB)

Instruments: QIAcuity Four and QuantStudio 5

Cycling: According to manufacturers' recommendations

Analysis: QIAcuity Software Suite 2.0.20 and QuantStudio Copy Caller Software (AB), based on reference sample with 2 copies

Recommendations for transfer of commercial qPCR assays on QIAcuity

Gene expression analysis with TaqMan assays (Applied Biosystems, Bio-Rad, IDT)

- Use assay concentration as provided and recommended by supplier
- Use the assays either with the QIAcuity Probe PCR Kit (cDNA) or with the QIAcuity OneStep Probe Kit (RNA); following the cycling and imaging recommendations described in the respective kit protocols

Copy number analysis (Applied Biosystems)

- Use assay concentration for the target of interest (FAM) as provided and recommended by supplier, reduce assay concentration of the reference target (VIC) to 0.25x of the concentration recommended.
- Use the assays with the QIAcuity Probe PCR following the cycling recommendations in the kit protocol

Single nucleotide polymorphism (SNP) analysis (Applied Biosystems)

- Use assay concentration as provided and recommended by supplier.
- Use the assays with the QIAcuity Probe PCR Kit following the cycling recommendations in the protocol **but reduce the number of cycles to 30**. This will prevent crosstalk of the VIC dye into the green channel and suppresses erroneous cross hybridization of the probes to the wrong SNP genotype.

Sample to Insight