

Pharmacogenomics Experiments

APPLICATION GUIDE

TaqMan[®] Genotyping and Copy Number Assays

for use with:

QuantStudio[™] 12K Flex Real-Time PCR System with OpenArray[™] Block
(with AccuFill[™] System)

TaqMan[®] OpenArray[™] Genotyping Plates

TaqMan[®] Genotyper Software

CopyCaller[™] Software

AlleleTyper[™] Software

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B.0	August 2014	<ul style="list-style-type: none">DNA sample preparation procedures in Chapter 4 updated for use of the MagMAX™ DNA Multi-Sample Ultra Kit. Users referred to a new Appendix A for complete procedures.“Perform analysis in TaqMan® Genotyper Software” topic in Chapter 5 reorganized for better workflow.Sample quantification procedure using the RNase P Detection Reagents Kit added to a new Appendix B.Corrections and clarifications made to Appendix C Troubleshooting.
A.0	January 2014	New document

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 **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](https://www.thermofisher.com/support).

Introduction

Pharmacogenomics (PGx) is the study of genetic variation as it relates to drug response. PGx studies involve testing samples for multiple variants in drug metabolism enzyme (DME) and transporter genes. This guide describes procedures for the sample-to-result PGx workflow solution using the QuantStudio™ 12K Flex Real-Time PCR System.

Variant testing uses TaqMan® SNP Genotyping Assay or TaqMan® Copy Number Assays targeting DME genes.

- The TaqMan® Drug Metabolism Genotyping Assay collection has ~2,700 assays that detect potentially causative polymorphisms in 221 drug metabolism enzyme and associated transporter genes.
- TaqMan® Copy Number Assays examine copy number variation (CNV) in DME genes.
- There are 7 million predesigned TaqMan® SNP Genotyping Assay and custom TaqMan® SNP Genotyping Assay available for other targets of interest.

Workflow for OpenArray™ plate PGx experiments

Chapter 3, Isolate DNA using the MagMAX™ DNA Multi-Sample Ultra Kit



(Optional) Appendix B, Preamplification of low-concentration gDNA



Chapter 4, Prepare and run OpenArray™ PGx SNP genotyping experiments

Chapter 6, Prepare, run, and analyze PGx copy number experiments



Chapter 5, Analyze OpenArray™ PGx SNP genotyping experiments



Chapter 7, Perform translation analysis in AlleleTyper™ Software



Note: See Appendix D for expected throughput rates of OpenArray™ plates and other system specifications.



Background and tools for assay selection

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DME allele nomenclature

SNPs within drug metabolism genes are often identified by their standardized allele name or star (*) allele nomenclature (Sim, 2010). Star alleles are gene-level haplotypes (a set of DNA polymorphisms that are often inherited together on the same chromosome). Often, these haplotypes have been associated with DME activity levels (for example, functional, decreased function, or nonfunctional variants). The combination of star allele haplotypes (that is, the diplotype) within a sample can be used to predict its drug metabolizer phenotype (for example, ultrarapid, extensive, intermediate, or poor). Genetic variants within a haplotype can include SNPs, InDels, and CNVs. The allele nomenclature for a specific gene family is maintained and standardized by an affiliated group of scientists that curate each site independently. This nomenclature is complicated, because many alleles contain more than one polymorphism (that is, they are haplotypes) and conversely, many polymorphisms can be associated with several alleles.

The star allele nomenclature contains the DME gene name, such as CYP2D6, followed by a numeric allele name, such as *3. A star allele conventionally contains at least one causative variant (for example, a frameshift mutation). Variants are given reference gene and/or cDNA coordinates, such as g.2549delA (full variant name: CYP2D6*3 g.2549delA). The causative star allele variant can be associated with other nucleotide variants in different haplotypes groups; such sub-alleles are denoted by letters following the numeric allele identifier (for example, *3A). On the Cytochrome P450 (CYP) Allele Nomenclature web site, the defining, causative variant for a star allele is often in bold font.

Note: *1 refers to the reference gene sequence, which produces an enzyme with normal function. The reference gene sequence is not necessarily equivalent to the reference genome assembly sequence, and it does not necessarily contain the major

allele for a given SNP (which can vary between populations, particularly for highly polymorphic SNPs).

The defining variant for a given DME gene star allele may be the only variant needed to identify that particular star allele. The defining allele is sometimes referred to as the “tag SNP”. Common allele names are provided for many DME variants in the PGx Common Markers file. See “The PGx Common Markers file” on page 26.

The DME Assay collection variants have been mapped for the genes having public allele nomenclature sites. This allele nomenclature is searchable on the DME assay product pages and in the downloadable TaqMan® Drug Metabolism Genotyping Assays Index file, available at www.thermofisher.com/pgx. The public allele nomenclature websites provide information on DME gene star allele haplotypes, the defining polymorphisms for these alleles, and links to the NCBI dbSNP website for variants having an reference SNP (refSNP) identifier, or rs ID.

Table 2 DME allele nomenclature websites

Gene family	Allele nomenclature website
CYP—Cytochrome P450 (CYP) genes	www.cypalleles.ki.se
NAT1 and NAT2—Arylamine N-Acetyltransferase genes	http://nat.mbg.duth.gr
UGT—UDP Glucuronosyltransferase genes	www.pharmacogenomics.pha.ulaval.ca/ugt-alleles-nomenclature

Note: Other DME gene variants have allele nomenclature reported in the literature, but no public nomenclature web site exists. For key variants, allele nomenclature is found in the Very Important PGx (VIP) gene summary pages on the Pharmacogenomics Knowledge Base web site www.pharmgkb.org.

Where possible, such allele nomenclature is provided for non-CYP, -NAT and -UGT variants in the PGx Common Markers file. See “The PGx Common Markers file” on page 26.

TaqMan® Drug Metabolism Genotyping Assays

The TaqMan® Drug Metabolism Genotyping Assay, a collection of approximately 2,700 assays, detect potentially causative SNP, MNP or InDel polymorphisms in 221 drug metabolism enzyme (DME) and associated transporter genes. The DME assays were designed using an optimized DME assay design algorithm that uses a high level of bioinformatics to ensure a lack of underlying polymorphisms and high target specificity (that is, gene family members and pseudogenes will not amplify). Assays to more difficult targets underwent manual design. All DME Assays underwent stringent wet-lab testing, including running with 180 unique DNA samples from four different populations (African American, Caucasian, Chinese, and Japanese).

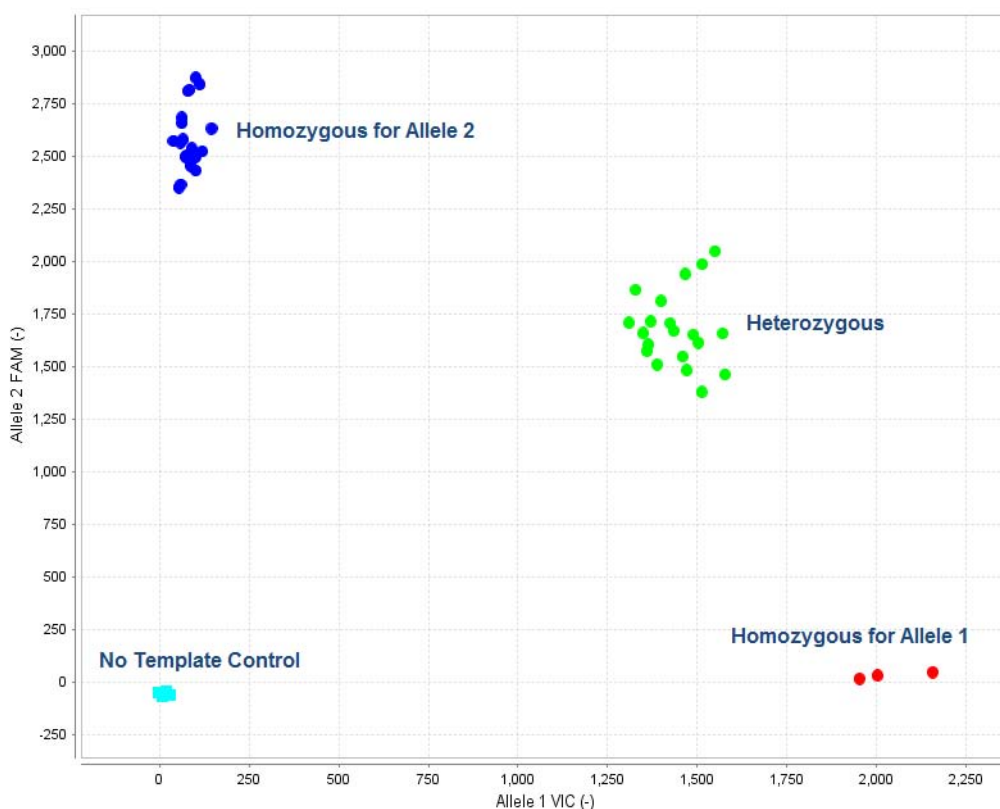
The TaqMan® SNP Genotyping Assays were designed using a related, highly validated SNP assay design algorithm. The SNP assays are functionally tested (that is, for amplification and clustering capabilities) when first manufactured on 20 unrelated gDNA samples from three populations (African American, Caucasian, and Japanese). In addition, over 300 high value TaqMan® DME and SNP assays were tested with 44 African American and Caucasian samples and synthetic templates representing each

genotype on OpenArray™ plates run on the QuantStudio™ 12K Flex Real-Time PCR System, to ensure assay performance on this platform.

All TaqMan® SNP Genotyping Assays, Drug Metabolism Genotyping Assays, and Custom SNP Genotyping Assays contain sequence-specific forward and reverse primers to amplify the polymorphic sequence of interest and two TaqMan® MGB probes with non-fluorescent quencher (NFQ):

- One probe labeled with VIC™ dye detects the Allele 1 sequence.
- One probe labeled with FAM™ dye detects the Allele 2 sequence.

TaqMan® DME and SNP Genotyping Assay data is analyzed by cluster plot analysis: FAM™ dye signal is plotted on the Y-axis, and VIC™ dye signal is plotted on the X-axis. Samples homozygous for the FAM™- or VIC™-labeled alleles form clusters along the Y- or X-axis, respectively, whereas heterozygous samples contain both FAM™ and VIC™ dye signal and will cluster roughly along the diagonal position between the homozygous clusters.



Genotyping assay context sequences

The reporter dye information for TaqMan® Drug Metabolism and SNP Genotyping Assays is represented in the assay context sequence, found at thermofisher.com and provided in the **Assay Information File (AIF)** that you can download from the web at www.lifetechnologies.com/OA-platefiles. The context sequence is the nucleotide sequence surrounding the SNP site. It is provided in the (+) genome strand orientation relative to the NCBI reference genome. The SNP alleles are included in brackets, where the order of the alleles corresponds to the association with probe reporter dyes, where [Allele 1 = VIC™ dye / Allele 2 = FAM™ dye].

Example: C__27102431_D0 assay

For C__27102431_D0 assay targets the CYP2D6*4 g.1846G>A SNP, rs3892097, and has the following context sequence:

AGACCGTTGGGGCGAAAGGGGCGTC [C/T] TGGGGGTGGGAGATGCGGGTAAGGG

The VIC™ dye probe is associated with the C allele and the FAM™ dye probe is associated with the T allele.

In this example, the SNP alleles (C/T) are the reverse complement of those given in the star allele nomenclature: CYP2D6*4 g.1846G>A. This is because the context sequence alleles are provided in the (+) reference genome strand orientation whereas the star allele nucleotide changes are provided with respect to the CYP2D6 gene reference sequence that maps to the (-) genome strand.

TaqMan® Drug Metabolism Genotyping Assay for genotyping triallelic SNPs

Several important DME gene variants are triallelic SNPs, wherein 3 bases occur at the same genomic location. Triallelic SNP targets can be interrogated using a pair of TaqMan® assays. Each assay contains one probe for the major SNP allele, which is labeled with the same reporter dye (VIC™ dye) in both assays, and one probe for one of the minor alleles, which is labeled with the second reporter dye (FAM™ dye). To generate accurate sample genotypes, the two assays must be run independently on the same panel of samples, and the resulting allelic discrimination plots must be analyzed in concert, comparing the genotype cluster positions from both assays to a map of the true sample genotypes. See Table 15, on page “Analysis guidelines: SNP genotyping assays for triallelic SNPs” on page 61 for analysis.

Table 3 TaqMan® Drug Metabolism Genotyping Assays to triallelic SNPs

Gene	rs ID	TaqMan® DME Assay	Allele name	Assay alleles [VIC™ /FAM™]
ABCB1	rs2032582	C_11711720C_30	ABCB1 c.3095G>T	C/A
		C_11711720D_40	ABCB1 c.3095G>A	C/T
CYP2C9	rs7900194	C__25625804_10	CYP2C9*8 c.449G>A	A/G
		C_25625804D_20	CYP2C9*27 c.449G>T	T/G
CYP2D6	rs5030865	C_30634117C_K0	CYP2D6*8 g.1758G>T	A/C
		C_30634117D_M0	CYP2D6*14 g.1758G>A	T/C
CYP1A1	rs41279188	C_30634152C_70	CYP1A1*5 g.2461C>A	G/T
		C_30634152D_80	CYP1A1*9 g.2461C>T	G/A
CYP2C8	rs72558195	C_72650009C_10	CYP2C8*7 c.556C>T	G/A
		C_72650009D_20	CYP2C8*8 c.556C>G	G/C

TaqMan® Drug Metabolism Genotyping Assays for genotyping adjacent SNPs

For certain DME targets, two SNPs are located adjacent to one another. This complicates the SNP genotype analysis because for each assay, the probes will fail to bind target sequences when the adjacent SNP is present. However, when the adjacent SNPs are present in only 3 haplotypes, these SNPs can be interrogated similarly to triallelic SNPs using two assays (see “TaqMan® Drug Metabolism Genotyping Assay for genotyping triallelic SNPs” on page 13).

Assay sets to two highly studied SNPs that have an adjacent, less frequently detected SNPs, are available. In each case, the minor alleles of each SNP are not found together in the same haplotype.

Table 4 DME Assays for genotyping adjacent SNPs

Gene	SNP ID	TaqMan® DME assay	Allele name
CYP2C19	rs4244285	C__25986767_70	CYP2C19*2 c.681G>A
	rs6413438	C__30634128_10	CYP2C19*10 c.680C>T
CYP2C9	rs1057910	C__27104892_10	CYP2C9*3 c.1075A>C
	rs56165452	C__30634131_20	CYP2C9*4 c.1076T>C

CYP2C19*2,*10 adjacent SNP assays

Only 3 haplotypes have been observed for the CYP2C19 *2 and *10 SNPs (www.cypalleles.ki.se/cyp2c19.htm): the rare *10 c.680T allele and the *2 c.681A do not occur on the same chromosome. Thus, these adjacent SNPs can be analyzed similarly as for triallelic SNPs. When the *2 assay is run on a sample containing a *10 allele, the probes will fail to detect the *10-containing allele; the converse is true when the *10 assay is run on a sample containing a *2 allele. The context sequences for each assay are shown below. After running paired assays for adjacent SNPs in separate reactions on the same gDNA samples, examine the cluster plots in TaqMan® Genotyper Software. Sample calls may need to be adjusted, and the results of each assay compared to determine the true sample genotype, as detailed in “Analysis guidelines: DME genotyping assays for genes in copy number variation regions” on page 61.

CYP2C19*2 c.681G>A C__25986767_70

TTCCCACTATCATTGATTATTTCCc [A/G] GGAACCCATAACAAATTACTTAAAA

CYP2C19*10 c.680 C>T C__30634128_10

TTTCCCACTATCATTGATTATTTCC [C/T] gGGAACCCATAACAAATTACTTAAA

*10 *2

TTTTCCCACTATCATTGATTATTTCC [C/T] [G/A] GGAACCCATAACAAATTACTTAAA

TaqMan® Copy Number Assays

Copy number variation must be assessed for DME genes known to exhibit copy number variation (see “Pre-tested TaqMan® Copy Number Assays for select DME genes” on page 16). TaqMan® Copy Number Assays are run simultaneously with a TaqMan® Copy Number Reference Assay in a duplex real-time polymerase chain reaction (PCR). TaqMan® Copy Number Assays detect the target gene or genomic sequence of interest and the TaqMan® Copy Number Reference Assay detects a sequence known to exist in two copies in a diploid genome (for example, the human RNase P H1 RNA gene). Relative quantitation (RQ) using the comparative C_t ($\Delta\Delta C_t$) method is used to determine the number of copies of the target sequence in each test sample.

- TaqMan® Copy Number Assays contain two primers and a FAM™ dye-labeled MGB probe to detect the genomic DNA target sequence.
- TaqMan® Copy Number Reference Assays contain two primers and a VIC™ dye-labeled TAMRA™ probe to detect the genomic DNA reference sequence.

TaqMan® Copy Number Assays are ordered as single tube assays (see “Single tube products and formats” on page 27) and run on 96-well and 384-well plates on Applied Biosystems™ Real-Time instruments, including the QuantStudio™ 12K Flex Real-Time PCR System. There are currently no copy number assays developed for the OpenArray™ plate.

DME genes and copy number variation

Several DME genes exhibit copy number variation (CNV) or other structural alterations due to recombination and gene conversions events between highly related loci (He, 2011).

For DME gene variants that are associated with copy number variation, run both DME genotyping assays and copy number assays to determine sample genotypes.

For DME assays that target a deleted or duplicated gene (for example, CYP2D6):

- samples that contain 0 copies of the gene will not amplify.
- samples with 1 or more gene copies that are homozygous for the SNP allele will cluster together
- samples with more than 2 gene copies that are heterozygous may run within the 2-copy heterozygous cluster or between it and one of the homozygous clusters.

To discern the sample genotype, copy number quantitation of the gene target must be done to determine which samples carry gene deletions or duplications. Additionally, use of multiple copy number assays for a gene may be required for hybrid gene analysis.

Note: The copy number assays are not SNP allele-specific and cannot be used to determine which SNP allele is duplicated when a sample is heterozygous and has three gene copies.

A protocol has been developed for determining allele-specific copy number of CYP2D6 alleles by digital PCR. For more information, see *CYP2D6 Allele-specific Copy Number Analysis Quick Reference* (Pub. No. MAN0011114).

Pre-tested TaqMan® Copy Number Assays for select DME genes

Pre-tested TaqMan® Copy Number Assays are available for the DME genes in CNV regions are shown in Table 5. These assays were run on 90 Coriell gDNA samples from African American and Caucasian populations (the same panel used for DME Assay validation).

Table 5 Pre-tested DME gene TaqMan® Copy Number Assays for CNV and hybrid gene analysis

Gene symbol	Assay ID	Gene location	Major alleles detected
CYP2D6	Hs00010001_cn ^[1]	Exon 9	CYP2D6 Deletion (*5); Duplications (for example, *1xN, *2xN, *4x2, *9x2, *10x2, *17xN, *35x2)
CYP2D6	Hs04083572_cn	Intron 2	CYP2D6 Deletion (*5); Duplications (for example, *1xN, *2xN, *4x2, *9x2, *10x2, *17xN, *35x2); 2D6/2D7 hybrid alleles with 2D7 exon 9 sequences (for example, *36, *83)
CYP2D6	Hs04502391_cn	Intron 6	CYP2D6 Deletion (*5); Duplications (for example, *1xN, *2xN, *4x2, *9x2, *10x2, *17xN, *35x2); 2D6/2D7 hybrid alleles with 2D7 exon 9 sequences (for example, *36, *83)
CYP2A6	Hs07545273_cn	Exon 1	CYP2A6 Deletion (*4); Duplication (*1x2)
CYP2A6	Hs07545274_cn	Intron 1	CYP2A6 Deletion (*4); Duplication (*1x2)
CYP2A6	Hs04488984_cn	Intron 2	CYP2A6 Deletion (*4); Duplication (*1x2); hybrid allele with 2A7 exons 1-2 and 2A6 exons 3-9 (*12)
CYP2A6	Hs07545275_cn	Intron 7	CYP2A6 Deletion (*4); Duplication (*1x2); hybrid allele with 2A7 exons 1-2 and 2A6 exons 3-9 (*12)
CYP2A7	Hs07545276_cn	Exon 1	CYP2A6 hybrid allele with 2A7 exons 1-2 and 2A6 exons 3-9 (*12)
CYP2A7	Hs04488016_cn	Intron 2	CYP2A6 hybrid allele with 2A7 exons 1-2 and 2A6 exons 3-9 (*12)
CYP2A7	Hs07545277_cn	Intron 7	No CYP2A6 alleles
CYP2E1	Hs00010003_cn	Promoter	CYP2E1 Duplication (*1x2)
GSTM1	Hs02575461_cn	Exon 1	GSTM1 Deletion (*0); Duplication
GSTT1	Hs00010004_cn	Intron 1	GSTT1 Deletion (*0)
SULT1A1	Hs03939601_cn	Intron 2	SULT1A1 Deletion; Duplication
UGT2B17	Hs03185327_cn	Exon 1	UGT2B17 in 150 kb Deletion (*2)

^[1] Assays in boldface type are those most frequently used for CNV and hybrid gene analysis.

CYP2D6 copy number variation and CYP2D6/CYP2D7 hybrid alleles

The CYP2D6 gene is the most highly polymorphic and complex of the DME genes. Over 100 star allele groups have been identified by the Cytochrome P450 Nomenclature Committee (www.cypalleles.ki.se). At least 4 of these groups give rise to alleles with substrate-dependent reduced enzyme activity, and more than 20 do not encode functional enzymes.

The CYP2D6 alleles are composed of SNP and InDel variants, CNVs, and hybrid alleles formed by recombination between CYP2D6 and highly related upstream pseudogene, CYP2D7, sequences. Individuals may carry null alleles (*5) or extra copies of CYP2D6 (*1, *2, *4, *9, *10, *17, *35). Some CYP2D6 alleles contain sequences derived from the highly homologous CYP2D7 pseudogene; for example, CYP2D6*36, as well as *4N, *57, and *83, contains a gene conversion to CYP2D7 sequences in exon 9 associated with negligible CYP2D6 enzyme activity (Gaedigk, 2006).

Three different copy number assays to CYP2D6 sequences are available for determining CYP2D6 gene copy number and to aid identification of some hybrid alleles (see “Pre-tested TaqMan® Copy Number Assays for select DME genes” on page 16):

Table 6 Pre-tested TaqMan® Copy Number Assays to CYP2D6: detection of CYP2D7 exon 9 conversion alleles

Gene	Assay ID	Gene location	CYP2D6 full length alleles	CYP2D6*36 [2D6/2D7 hybrid] ^[1]	CYP2D7 full length alleles
CYP2D6	Hs00010001_cn	Exon 9	Yes	No	No
	Hs04083572_cn	Intron 2	Yes	Yes	No
	Hs04502391_cn	Intron 6	Yes	Yes	No

^[1] Other alleles with conversions to CYP2D7 sequences in exon 9: *4N, *57, *83

The primary copy number assay for CNV analysis is the exon 9 Hs00010001_cn assay, which predominantly detects full-length CYP2D6 alleles and not hybrid alleles containing the exon 9 conversion to CYP2D7 sequences. Usually it is not necessary to detect these nonfunctional hybrid alleles as they not contribute to CYP2D6 metabolizer status (see category A samples in Table 7).

The intron 2 and/or intron 6 assays should be run, in addition to the exon 9 assay, when information for both CYP2D6 and hybrid alleles is needed (see Table 7), for example, to characterize samples that are:

- Category B—0 copies for the exon 9 assay, but show amplification by SNP assays upstream of exon 9 may carry a *36 allele.
- Category C—single copy for the exon 9 assay, but are heterozygous for SNPs upstream of exon 9 may carry a *36 allele.
- Category D—single copy for the exon 9 assay, but 5’ located SNP assays do not amplify and may carry a *13 allele.
- Category E—2 copies for the exon 9 assay and carry a *13 allele. Such samples, which are relatively rare, are difficult to detect unless all 3 CYP2D6 CNV assays are routinely run.

Table 7 Example CYP2D6 SNP and copy number assay results for samples carrying hybrid alleles with CYP2D7 sequences

Category	Diplotype	SNP assay results (cDNA alleles)				copy number assay results			Translation with exon 9 result only ^[1]
		100C>T	1846G>A	2850C>T	4180G>C	exon 9	intron 6	intron 2	
A	*10/*36	T/T	G/G	C/C	C/C	1	2	2	*5/*10
A	*2/*36- *10	C/T	G/G	C/T	C/C	2	3	3	*2/*10
A	*4/*36- *10	T/T	G/A	C/C	C/C	2	3	3	*4/*10
A	*1/*4N- *4	C/T	G/A	C/C	G/C	2	3	3	*1/*4
B	*5/*36	T/T	G/G	C/C	no amp	0	1	1	und (= *5/*5)
C	*4/*36	T/T	G/A	C/C	C/C	1	2	2	und (= *4/*5)
C	*1/*36- *36	C/T	G/G	C/C	G/G	1	3	3	und (= *5/*1)
D	*5/*13F	no amp	no amp	no amp	G/G	1	0	0	und (= *5/*5)
E	*10/*13E	T/T	G/G	C/C	C/C	2	1	1	*10/*10

^[1] und= undetermined

See The Human Cytochrome P450 (CYP) Allele Nomenclature Database CYP2D6 web page (www.cypalleles.ki.se/cyp2d6.htm) or the PharmGKB web page (www.pharmgkb.org), plus associated references, for more details about these alleles.

IMPORTANT! Always use the CYP2D6 exon 9 copy number assay to detect true gene duplication events. CYP2D6 intron 2 or intron 6 copy number assays should not be used alone to detect CYP2D6 duplications as they will also detect nonfunctional hybrid alleles that do not represent duplications and that do not contribute to CYP2D6 metabolizer status.

CYP2A6 copy number variation and CYP2A6/CYP2A7 hybrid alleles

CYP2A6 alleles defined by the Cytochrome P450 Nomenclature Committee (www.cypalleles.ki.se) include at least four non-functional alleles and several reduced function alleles. The genotyping of CYP2A6 alleles is complicated by the presence of copy number variant deletion (*4) and duplication (*1) alleles, and hybrid alleles formed by recombination with upstream pseudogene CYP2A7 sequences, for example, the reduced function *12 allele contains exons 1-2 of CYP2A7 origin and exons 3-9 of CYP2A6 origin (Oscarson, 2002).

Several copy number assays to CYP2A6 and CYP2A7 sequences are available for examining copy number variants and hybrid alleles (see “Pre-tested TaqMan® Copy Number Assays for select DME genes” on page 16 for the complete list).

Table 8 Pre-tested TaqMan® Copy Number Assays to CYPAD6 and CYP2A7: detection of hybrid alleles

Gene	Assay ID	Gene location	CYP2A6 full length alleles	CYP2A6*12 [2A7/2A6 hybrid]	CYP2A7 full length alleles
CYP2A6	Hs07545273_cn	Exon 1	Yes	No	No
	Hs07545274_cn	Intron 1	Yes	No	No
	Hs04488984_cn	Intron 2	Yes	Yes	No
	Hs07545275_cn	Intron 7	Yes	Yes	No
CYP2A7	Hs07545276_cn	Exon 1	No	Yes	Yes
	Hs04488016_cn	Intron 2	No	Yes	Yes
	Hs07545277_cn	Intron 7	No	No	Yes

At least two assays are required to detect copy number variation in CYP2A6.

- The CYP2A6 intron 7 assay can be used to detect *4 deletion and *1 duplication alleles. This assay will amplify both full length CYP2A6 and the partially active CYP2A6/CYP2A7 hybrid allele, CYP2A6*12, but will not be able to discern them.
- The intron 1 assay can also be used to detect *4 deletion and *1 duplication alleles. This assay will amplify full length CYP2A6 but will not be able to amplify the CYP2A6*12 hybrid allele.
- If both CYP2A6 assays are run, samples containing *12 alleles can be distinguished from those containing full-length alleles. For example, a *1/*12 sample will give 2 copies using the intron 7 assay and 1 copy using the intron 1 assay, whereas a *1/*1 sample will give 2 copies with both assays.

Additionally, CYP2A7 assays can be used to corroborate the presence of CYP2A6*12 alleles: The CYP2A7 exon 1 assay will amplify the hybrid CYP2A6*12 allele, whereas the intron 7 assay will not amplify intact CYP2A6 or the CYP2A6*12 allele.

**GSTM1 and GSTT1
DME assays and
CNV**

The GSTM1 and GSTT1 genes have a very high frequency of deletion and are entirely missing in a substantial number of individuals in multiple populations. DME genotyping assays to variants within these genes will not amplify samples that are homozygous for the gene deletion; samples that are heterozygous for the deletion will run as a homozygous sample.

Special assays**Gender assays**

Gender-specific assays in PGx studies can aid sample tracking. The TaqMan® SNP Genotyping Assay, C_990000001_10, targets a gender-specific polymorphic region in the amelogenin gene that is commonly used in forensic sex determination tests. The VIC™ dye probe detects a 6 base deletion which occurs in the X-specific amelogenin gene, whereas the FAM™ dye probe detects Y-chromosome sequences. In genotyping experiments, male samples run in the heterozygous cluster position and female samples run in the VIC™ homozygote cluster. Some males lack the Y-specific amelogenin gene, and will type as female. Run the C_990000001_10 assay in combination with a Y-chromosome assay to identify any mistyped samples. For more information, go to www.cstl.nist.gov/strbase/Amelogenin.htm.

An example of a genotyping bar code Y-chromosome assay is the C__1083232_10 assay to the polymorphic rs2032598 SNP in USP9Y. Only male samples amplify with this assay; female samples will cluster with the no template controls (NTCs).

**Clinical research
targets**

TaqMan® SNP Genotyping Assays for non-ADME PGx targets and clinical research targets are often included in PGx studies. These assays are found within the predesigned TaqMan® SNP Genotyping Assays collection and are searchable on the Thermo Fisher Scientific web site by NCBI dbSNP rs ID. Note that common names for disease-associated alleles (for example, Factor V Leiden) are not yet searchable terms. Public web sites that can be used to identify the rs ID for common disease alleles include:

- Pharmacogenomics Knowledge Base (PharmGKB): www.pharmgkb.org
- Online Mendelian Inheritance in Man™ (OMIM®): <http://omim.org/>
- SNPedia (a wiki investigating human genetics): <http://snpedia.com/>

In addition, TaqMan® SNP Genotyping Assays for commonly tested clinical research targets are included in the PGx Common Markers file. Download the PGx Common Markers file from www.thermofisher.com/pgx.

**PGx targets not
amenable to
TaqMan® design**

TaqMan® SNP Genotyping Assays are an ideal technology for interrogation of most DME and clinical research target polymorphisms, offering highly specific target amplification and allele discrimination. However, there are some polymorphisms that are not well-suited for TaqMan® Assay development. These include targets that:

- Reside in highly polymorphic genomic regions (polymorphisms interfere with amplification in some samples)
- Share high sequence identity with another genomic region (base differences are not available for specific assay development)
- Are microsatellite polymorphisms
- Are base deletions within a homopolymer sequence

The PGx Common Markers file (www.thermofisher.com/pgx) contains a list of commonly requested DME and clinical research targets that are not good candidates for TaqMan® Assay design and suggestions for alternative technologies to use (see the 'No TaqMan Assays' tab).

Custom TaqMan® SNP Genotyping Assays

Targets of interest that are not covered by the current TaqMan® SNP Genotyping Assay collection can be submitted for Custom TaqMan® SNP Genotyping Assays design.

The Custom TaqMan® Assays Design Tool (CADT) is available at www.thermofisher.com/taqmansnpdesign.

Order Custom TaqMan® SNP Genotyping Assays by first entering a sequence with the SNP in brackets, for example [A/G], then submitting the chosen target sites for assay design. See www.thermofisher.com/taqmansnpdesign.

CADT is used to design assays targeting biallelic SNPs or insertion/deletion polymorphisms and multi-nucleotide polymorphisms (MNP) that are 6 bases or fewer in length.

Sequences must be SNP and repeat-masked before submission to CADT. Additionally, the genome-uniqueness for assays must first be established, because custom assays are not compared to the genome (for example, by BLAT or BLASTn) to determine target specificity. Do not submit targets from the “No TaqMan Assays” tab (see “PGx targets not amenable to TaqMan® design” on page 20), as assays can be designed that do not function properly.

For targets that present assay design challenges, contact our fee-for-design custom assay design service at custom.solutions@lifetech.com.

TaqMan® Copy Number Assays

Targets of interest that are not covered by the extensive human TaqMan® Copy Number Assays collection can be submitted to TaqMan® Copy Number Assays design. The GeneAssist™ Copy Number Assay Workflow Builder is available at www.thermofisher.com/order/custom-genomic-products/tools/copy-number-variation.

Tools for finding PGx TaqMan® DME, SNP, and Copy Number Assays

- Assay search tool available at www.thermofisher.com/ordertaqman
- TaqMan® Drug Metabolism Genotyping Assays Index available at www.lifetechnologies.com/taqmanandme
- PharmaADME Core Marker Set available at http://tools.lifetechnologies.com/content/sfs/brochures/cms_082106.xls
- PGx Common Markers file available at www.thermofisher.com/pgx

Assay Search tool

The Thermo Fisher Scientific web site provides an easy-to-use assay search tool to aid selection of TaqMan® DME, SNP and Copy Number Assays. The search tool can be accessed from the Real-Time PCR Assays page or any of the specific product pages at thermofisher.com.

Search for TaqMan® SNP Genotyping Assays

1. Go to www.thermofisher.com/ordertaqman.
2. Select **SNP Genotyping**.

Assay Search Tool - Find & Buy Your Single Tube TaqMan® Assays:

What type of experiment are you conducting?

What type of assay do you want?

What species do you want to target? (Select one or more)

Enter target information
 e.g., Assay ID, dbSNP ID, Gene Symbol, hCV ID

 Include 10kb Flanking Region

What chromosome position are you interested in?
 Number:
 Position/Start:
 Position/Stop:

3. Select **All SNP Genotyping** ▶ **Human** (includes the DME Assays) or just the validated **Drug Metabolism Assays** collection.
4. Enter **target information**, then click **Search** to search the complete assay set. Optionally, specify other search terms (including allele nomenclature, rs ID and Assay ID) or specify chromosome position. Use the **Enter/Upload Multiple Targets** option to specify multiple search terms.

Note: If allele nomenclature is used as a search term (for example, CYP2D6*4), all DME assays to variants within all associated sub-alleles will be returned. If needed, review the variants on the associated allele nomenclature website to

determine which are important to evaluate in your study (for example, only the allele-defining variants may be of interest).

IMPORTANT! Read any Important Information notes associated with the assay (select **Important Information** beside the Assay ID on the assay search results bar to open a pop-up window). These notes provide information required for making ordering decisions. For example, a copy number assay may be required in addition to the DME assay for sample genetic analysis, or the DME assay may be one of a pair of assays interrogating a triallelic SNP.

5. To review the information for each assay, click **View Assay on Map**, **View Details**, and **View Allele Frequency** (if available).

Note the following:

- Read any **Important Information** notes associated with the assay (select **Important Information** next to the Assay ID). Notes provide information required to make ordering decisions and analyze data.
 - The annotation for triallelic SNP assays is associated with the SNP ID and not to the assay. Review the **Important Information** or the assay context sequence to determine the target alleles of each assay.
 - Click **View Details** to review allele nomenclature for CYP, UGT, and NAT genes.
6. To prepare a list of assays for ordering, select the assays of interest, then click **Export** (at the top of the page).
The exported results contain the Assay ID and annotations (catalog number, SNP, gene, context sequence, genomic location, and allele nomenclature).

Search for TaqMan® Copy Number Assays

1. Go to www.thermofisher.com/ordertaqman.
2. Select **Copy Number** ▶ **Human**.

Assay Search Tool - Find & Buy Your Single Tube TaqMan Assays:

What type of experiment are you conducting?

Gene Expression
 SNP Genotyping
 Copy Number
 siRNA

MicroRNA
 Mutation Detection
 Antibodies
 Engineered Cell Models

Which Copy Number product(s) are you interested in using?

All Copy Number Assays
 Markers & Reporters

What species do you want to target? (Select one or more)

[Hs] Human
 [Mm] Mouse
 All

Enter target information

e.g., Assay ID, Gene Symbol/Name, Refseq, DGV

What chromosome position are you interested in?

Number

Position/Start

Position/Stop

-

3. Enter **target information**. Search terms include gene symbol, Assay ID, and Database of Genomic Variants (DGV) variation IDs.
 - Under **Narrow Your Results** (to the left of the results), select the **Gene name** and **Pre-tested Assay filters**.
 - Use the **Enter/Upload Multiple Targets** option to specify multiple search terms.
 - Specify chromosome position.

Note: Copy Number Assays must be run in duplex PCR with a TaqMan® Copy Number Reference Assay; RNase P (default assay) and TERT (alternate assay) assays are available.

IMPORTANT! Read any Important Information notes associated with the assay (select **Important Information** beside the Assay ID on the assay search results bar to open a pop-up window). These notes provide information required for making ordering decisions. For example, information on the particular star alleles that an assay detects.

4. Click **Search** to search the complete assay set.

5. When applicable, narrow results by selecting **Pre-Tested Assays** to view assays pre-tested on 90 Coriell gDNAs.
6. Review the information for each assay: click **View Assay on Map** and **View Details**.
Note the following:
 - Read any **Important Information** notes associated with the assay (click the **Important Information** box next to an Assay ID). Notes provide information required to make ordering decisions, then analyze data.
 - **View Details** provides target location within gene and targeted known copy number variants.
7. If you need a list of assays for ordering, select the assays of interest, then click **Export** (at the top of the page).
The exported results contain the assay ID and annotations (catalog number, gene, genomic location, and DGV targets).
Note: TaqMan® Copy Number Assays must be run in duplex PCR with a TaqMan® Copy Number Reference Assay: RNase P (default assay) and TERT (alternate assay) assays are available.

The TaqMan® Drug Metabolism Genotyping Assays Index

The TaqMan® Drug Metabolism Genotyping Assays Index contains a comprehensive list of the DME assays along with the annotations listed below. This file can facilitate looking for DME assays to polymorphisms of interest given the extensive annotation information within it, which includes:

- Gene symbol and name
- NCBI SNP reference (if applicable)
- Polymorphism (for example, A/G)
- Amino acid change (if applicable)
- Allele nomenclature (if available)
- Polymorphism (for example, A/G)
- SNP type (for example, missense mutation)
- Context sequence [VIC™/FAM™] (in (+) strand orientation with SNP alleles in brackets)
- Applied Biosystems™ minor allele frequency data (Caucasian, African American, Japanese, Chinese populations)

The PharmaADME Core Marker Set

The PharmaADME consortium (www.PharmaADME.org) created a consensus list of known and putative functional variants in key genes involved in the absorption, distribution, metabolism, and excretion (ADME) of drugs. The PharmaADME Core Marker Set contains a list of variants considered most likely to impact drug metabolism, and is composed of 184 variants in 33 key ADME genes.

Thermo Fisher Scientific developed TaqMan® Assays to the PharmaADME Core Markers (> 95% coverage). This assay set is comprised of both TaqMan® DME and Copy Number Assays to 172 SNP, InDel, and CNV targets:

- 164 DME assays include 10 assays to genotype 5 triallelic SNPs (paired assays)
- 10 copy number assays cover deletions and duplications in 6 total DME genes

The PGx Common Markers file

Thermo Fisher Scientific has pretested over 300 TaqMan® DME and SNP Assays to highly studied, important DME and other PGx gene variants on OpenArray™ plates run on the QuantStudio™ 12K Flex Real-Time PCR System. All assays on this list were tested on this system with 44 Coriell gDNAs (22 each African American and Caucasian samples, a subset of the DME Assay 90 sample validation panel) and most were also tested with synthetic plasmid constructs representing each genotype. The PGx Common Markers file contains a list of the most commonly requested assays from this set.

The file includes common allele names, context sequences, and other useful annotations.

Note: A presentation called *TaqMan® Drug Metabolism Genotyping Assays on OpenArray™ Plates* contains screen shots of the test data for the most commonly requested PGx Marker Assays (download from www.thermofisher.com/pgx).

Plate products and formats

Fixed TaqMan® OpenArray™ PGx panel plates

There are two fixed TaqMan® OpenArray™ PGx panels available for use on the QuantStudio™ 12K Flex Real-Time PCR System:

- TaqMan® OpenArray™ PGx Panel, QuantStudio™ 12K Flex (Cat. No. 4475395)
- TaqMan® OpenArray™ PGx Express Panel, QuantStudio™ 12K Flex (Cat. No. 4488847)

Order custom OpenArray™ plates

1. Go to www.thermofisher.com/order/custom-array/.
2. For array type, select **TaqMan® OpenArray™ Genotyping Plates**.
3. Click **View Layout** to display the assay format in a plate.
4. Click **Select** to configure a plate.

Array name*	Array ID	Array type	Format	Unique Targets	Filled	Invalid	Empty
Name your array	-	TaqMan® OpenArray® Genotyping Plates	64	0	0	0	64

Select Edit Move Export Help Save Your Array Save A Copy...

Click to select assays | Click & drag to move assays | Ctrl+C to copy an assay | Ctrl+V to paste an assay

Display Assay Target

Assay Target	1	2	3	4	5	6	7	8
a1	●	○	○	○	○	○	○	○
a2	○	○	○	○	○	○	○	○
a3	○	○	○	○	○	○	○	○
a4	○	○	○	○	○	○	○	○
a5	○	○	○	○	○	○	○	○
a6	○	○	○	○	○	○	○	○
a7	○	○	○	○	○	○	○	○
a8	○	○	○	○	○	○	○	○
b1	○	○	○	○	○	○	○	○
b2	○	○	○	○	○	○	○	○
b3	○	○	○	○	○	○	○	○
b4	○	○	○	○	○	○	○	○

Sub Array A1

Filled 0

Invalid 0

Empty 64

For optimal results, it is recommended to avoid these locations. These positions may produce variable performance.

If there are any questions, please contact QuantStudioFrontDesk

5. Click **Import Your Assay List**, then provide assay information.
 - Under **Upload a list of Assay IDs**, click **Choose File**, then select a tab-delimited text file (.txt) containing Assay IDs.
or
 - Under **Enter a list of Assay IDs**, paste the Assay IDs, then click **Import Entered List**.
6. Follow the screen instructions to configure the assays on the plate.
7. (Optional) Click **Save Your Array** at any time to save the array configuration to your Thermo Fisher Scientific account.
8. When the plate is configured, click **Complete Your Design**, then follow the screen instructions to complete the order.

Single tube products and formats

Order single-tube TaqMan® assays

Order single-tube assays in two ways:

- Search for TaqMan® Assays, add assays to the shopping cart, then complete the order.
- If you have already obtained catalog numbers and IDs (Assay IDs), click **Quick Order** at the top of any page at thermofisher.com.



Formats for DME and CNV assays

TaqMan® SNP Genotyping Assays

Table 9 Single-tube TaqMan® SNP Genotyping Assays

Item	Scale	Number of reactions		Assay mix formulation	Cat. No.	
		384-well	96-well		Human	Non-human
Predesigned SNP	Small	1,500	300	40X	4351379	N/A
	Medium	5,000	1,000		4351376	
	Large	12,000	2,400	80X	4351374	
DME	Small	750	150	20X	4362691	
Custom SNP	Small	1,500	300	40X	4331349	4332077
	Medium	5,000	1,000		4332072	4332075
	Large	12,000	2,400	80X	4332073	4332076

Note: For more detailed instructions on running SNP genotyping experiments with single tube assays, see the *TaqMan® SNP Genotyping Assays User Guide* (Pub. No. MAN0009593).

DME assays are available only as small scale inventoried product. Predesigned and custom SNP assays are made-to-order and are available in multiple scales.

Assays with human part numbers undergo functional testing on a panel of 20 unrelated Coriell cell line gDNA samples from 3 populations (African American, Caucasian, and Japanese) before shipment upon first order.

Custom non-human part numbers can be applied to assays for human targets that would fail the human assay functional test (for example, Y chromosome SNP assays fail as 8 of 20 samples are female and will not amplify; GSTT1 and GSTM1 SNP assays will also fail due to the high frequency of gene deletion).

TaqMan® Copy Number Assays

Table 10 Made-to-order assays

Scale	Number of reactions		Assay mix formulation	Cat. No. (Human)		
	384-well	96-well		Pre-designed assays	Custom Plus assays	Custom assays
Small	720	360	20X	4400291	4442487	4400294
Medium	1,500	750		4400292	4442520	4400295
Large	5,800	2,900	60X	4400293	4442488	4400296

Table 11 Inventoried assays

Item	Number of reactions		Assay mix formulation	Cat. No.
	384-well	96-well		
TaqMan® Copy Number Reference Assay RNase P (1 tube)	1,500	750	20X	4403326
TaqMan® Copy Number Reference Assay RNase P (4 tubes)	6,000	3,000	20X	4403328
TaqMan® Copy Number Reference Assay TERT (1 tube)	1,500	750	20X	4403316
TaqMan® Copy Number Reference Assay TERT (4 tubes)	6,000	3,000	20X	4403315

Options for master mixes

TaqMan® OpenArray™ Genotyping Master Mix is required for use with OpenArray™ plates. TaqPath™ ProAmp™ Master Mix is recommended for optimal performance with TaqMan® Copy Number Assays for pharmacogenomics applications.

Item	Use	Cat. No.
TaqMan® OpenArray™ Genotyping Master Mix	OpenArray™ genotyping	4404846
TaqPath™ ProAmp™ Master Mix	Copy number experiments	A30865 (ROX™)

3

Isolate DNA using the MagMAX™ DNA Multi-Sample Ultra Kit

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- Required materials not supplied 31
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- Isolate DNA from whole blood 37
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- Normalize DNA samples for copy number analysis 41

Kit contents and storage

Component	Cat. No. A25597 ^[1] (500 rxns)	Cat. No. A25598 ^[2] (2,500 rxns)	Storage
Proteinase K ^[3]	4 mL	5 × 4 mL	-25°C to -15°C
PK Buffer	96 mL	5 × 96 mL	15°C to 30°C
Multi-Sample DNA Lysis Buffer	100 mL	5 × 100 mL	
RNase A ^[4]	2 × 1.25 mL	10 × 1.25 mL	-25°C to -15°C
DNA Binding Beads ^[3]	8 mL	5 × 8 mL	2°C to 8°C
Nuclease-free Water	100 mL	5 × 100 mL	15°C to 30°C
Wash Solution 1 Concentrate	80 mL ^[5]	5 × 80 mL ^[5]	
Wash Solution 2 Concentrate	162 mL ^[5]	5 × 162 mL ^[5]	

Component	Cat. No. A25597 ^[1] (500 rxns)	Cat. No. A25598 ^[2] (2,500 rxns)	Storage
DNA Elution Buffer 1	25 mL	5 × 25 mL	15°C to 30°C
DNA Elution Buffer 2	25 mL	5 × 25 mL	

^[1] Also available as Cat. No. A25919, containing Cat. No. A25597 with **one additional tube** each of Proteinase K (4 mL) and DNA Binding Beads (8 mL).

^[2] Also available as Cat. No. A25920, containing Cat. No. A25598 with **5 additional tubes** of Proteinase K (4 mL each) and **5 additional tubes** of DNA Binding Beads (8 mL each).

^[3] Proteinase K is also available as Cat. no. A25561 and DNA Binding Beads are also available as Cat. No. A25562.

^[4] Not used for DNA isolation from buccal swabs.

^[5] Final volume; see “Before first use of the kit” on page 34.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**.
 MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Table 12 Required materials and equipment not included with the kit

Item	Source
One of the following instruments	
<i>(Recommended)</i> KingFisher™ Flex Magnetic Particle Processor	5400630
MagMAX™ Express-96 Magnetic Particle Processor	— ^[1]
Equipment	
Plate shaker, capable of shaking plates at a minimum of 900 rpm	88880023
Analog Vortex Mixer	Fisher Scientific 02-215-365
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
<i>(Optional)</i> Magnetic Stand-96	AM10027
Plates and combs	
Deep Well Plates, one of the following:	
KingFisher™ Flex Microtiter Deepwell 96 plates, sterile	95040460
MagMAX™ Express-96 Deep Well Plates	4388476
Standard Well Plates, one of the following:	
KingFisher™ 96 KF microplates	97002540
MagMAX™ Express-96 Standard Plates	4388475

Item	Source
Tip Combs, one of the following:	
KingFisher™ 96 tip comb for DW magnets	97002534
MagMAX™ Express-96 Deep Well Tip Combs	4388487
Other consumables	
MicroAmp™ Clear Adhesive Film	4306311
RNase-free Microfuge Tubes (2.0 mL)	AM12425
Conical tubes (15 mL)	AM12500
Conical tubes (50 mL)	AM12502
Aerosol-resistant pipette tips	MLS
Reagent reservoirs	MLS
<i>(Optional)</i> Paraffin film	MLS
Reagents	
Ethanol, 200 proof (absolute)	MLS
Isopropanol, 100% (molecular grade or higher)	MLS

^[1] Not available for sale.

Table 13 Additional materials and equipment required for processing buccal swabs

Item	Source
Laboratory incubator with slatted shelves, capable of maintaining 65°C	MLS
One of the following types of buccal swabs, or equivalent buccal swabs with foam tips:	
Puritan™ PurFlock™ Ultra Flocked Swabs	Fisher Scientific 22-025-192
Puritan™ HydraFlock™ Swabs, standard tip	Puritan 25-3306-H
Sterile Foam Tipped Swabs	Puritan 25-1506 1PF
4N6FLOQSwabs™, regular tip	4473979
<i>(Optional)</i> Proteinase K, 500 reactions (4 mL)	A25561
<i>(Optional)</i> DNA Binding Beads, 500 reactions (8 mL)	A25562

Table 14 Additional materials and equipment required for processing whole blood samples

Item	Source
Laboratory incubator with slatted shelves, capable of maintaining 65°C	MLS
<i>(Optional)</i> Proteinase K, 500 reactions (4 mL)	A25561
<i>(Optional)</i> DNA Binding Beads, 500 reactions (8 mL)	A25562

Download the KingFisher™ Flex program (if needed)

The program required for this protocol is not pre-installed on the KingFisher™ Flex Magnetic Particle Processor.

1. On the MagMAX™ DNA Multi-Sample Ultra Kit web page, scroll down to the **Product Literature** section.
2. Click **A25597_Blood_Buccal** to download the program to your computer.
3. See *Thermo Scientific™ KingFisher™ Flex User Manual* (Cat. No. N07669) and *BindIt™ Software User Manual* (Cat. No. N07974) for instructions for installing the program on the instrument.

Set up the sample layout

Set up the sample plate layout using either of the following tools, which provide sample tracking from the 96-well plate used for DNA isolation to the 96-well sample plate .csv file used for import into OpenArray™ Sample Tracker Software.

Include at least 2 no template controls (NTCs) per plate.

Tool	Obtain from	Description
OA_Genotyping_CalcSheet	thermofisher.com/oaqrc	Contains a sample layout tab, additional sample tracking tabs, and instructions for using an adjustable pipettor to transfer samples from the 96-well plate to the 384-well sample plate.
96-well Sample Plate 1.csv template	On the computer on which the Sample Tracker Software is installed: C:\Program Files\Applied Biosystems\Sample Tracking Utility\examples	Contains a sample layout tab.

Isolate DNA from buccal swabs

This section contains brief procedures. For detailed information, refer to *MagMAX™ DNA Multi-Sample Ultra Kit (human buccal swabs) User Guide* (Pub. No. MAN0010293).

Refer to *Best Practices for Collection of Buccal Swabs Quick Reference (Genotyping Experiments)* (Pub. No. MAN0014348) for sample collection instructions.

Guidelines for buccal swab preparation

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Equilibrate buccal swabs to room temperature to maximize DNA recovery.
- Remove the buccal swabs from the lysate:
 - Option 1 (Preferred): Transfer the lysate to a new plate. This option eliminates contamination risks and saves time. To transfer lysates, set a multi-channel micropipettor to ~300 µL and transfer one row at the time. Each well should contain 200–250 µL after transfer.
 - Option 2: Remove the swabs from the plate using forceps. Rinse the forceps in 70% ethanol between samples, to prevent cross-contamination. Press the swabs against the side of the well when removing them, to prevent sample loss.
- Cover the plate during the incubation and shaking steps to prevent spill-over and cross-contamination. The same MicroAmp™ Clear Adhesive Film can be used throughout the procedure, unless it becomes contaminated.
- If you use a plate shaker other than the recommended shaker, verify that:
 - The plate fits securely on your plate shaker.
 - The recommended speeds are compatible with your plate shaker. Ideal shaker speeds allow for thorough mixing without splashing.
- Per-plate volumes for reagent mixes are sufficient for one plate plus overage. To calculate volumes for other sample numbers, refer to the per-well volume and add 5% overage.
- (Optional) To prevent evaporation and contamination, cover the prepared processing plates with paraffin film until they are loaded into the instrument.

(Optional) Guidelines for improving yields

- This procedure is optimized for processing of one swab per well. It is possible to process two swabs in one well when a higher concentration of DNA is required.
- If the DNA yield is lower than expected, extend the Proteinase K digestion to 45 minutes.
- To further improve recovery, digest with Proteinase K overnight at 50°C.

Before first use of the kit

Prepare the Wash Solutions from the concentrates:

- Add 25 mL of isopropanol to Wash Solution 1 Concentrate, mix, and store at room temperature.
- Add 132 mL of ethanol to Wash Solution 2 Concentrate, mix, and store at room temperature.

Before each use of the kit

- Preheat the incubator to 65°C.
- Vortex DNA Binding Beads thoroughly, then combine with Nuclease-free Water according to the following table.

Component	Volume per well	Volume per plate
DNA Binding Beads	16 µL	1.6 mL
Nuclease-free Water	24 µL	2.4 mL
Total DNA Binding Bead Mix	40 µL	4 mL

Store DNA Binding Bead Mix at room temperature for up to 24 hours.

Digest the samples with Proteinase K

Ensure that the incubator is preheated to 65°C.

1. Place the swab, swab-head down, in a deep-well plate (one per well).
If two swabs were collected, store the second swab as a backup sample.
2. Break enough of the stick off the swabs so that the swabs sit in the wells without protruding.
3. Prepare sufficient PK Mix according to the following table, then invert several times to thoroughly mix components.

IMPORTANT! Prepare the PK Mix just before use. Do not place the PK Buffer or the PK Mix on ice, to avoid precipitation.

Component	Volume per well	Volume per plate
Proteinase K	8 µL	800 µL
PK Buffer	192 µL	19.2 mL
Total PK Mix	200 µL	20 mL

4. Add 200 µL of PK Mix to each sample well of a deep-well plate (PK Plate) containing a swab.

IMPORTANT! Do not touch any part of the swab with the pipet tip when pipetting the PK Mix in the sample wells.

5. Seal the plate with a clear adhesive film, then shake the sealed plate for 5 minutes at 900–950 rpm.
6. Incubate for 20–45 minutes at 65°C.

IMPORTANT! Arrange plates in the incubator to allow adequate flow around the plate wells, to ensure that samples quickly reach and maintain the incubation temperature.

Set up the processing plates

1. While the samples are incubating at 65°C, set up the Wash, Elution, and Tip Comb Plates outside the instrument as described in the following table.

Plate ID	Plate position ^[1]	Plate type	Reagent	Volume per well
Wash Plate 1	2	Deep Well	Wash Solution 1	150 µL
Wash Plate 2	3	Deep Well	Wash Solution 2	150 µL
Wash Plate 3	4	Deep Well	Wash Solution 2	150 µL
Elution Plate ^[2]	5	Standard	DNA Elution Buffer 1	50 µL
Tip Comb	6	Deep Well	Place a tip comb in the plate.	

^[1] Position on the instrument

^[2] The instrument prompts the user to add DNA Elution Buffer 2 to the Elution Plate, after incubation with DNA Elution Buffer 1.

2. (Optional) To prevent evaporation and contamination, cover the prepared processing plates with paraffin film until they are loaded into the instrument.

Add Multi-Sample DNA Lysis Buffer, isopropanol, and DNA Binding Bead Mix

1. (Optional) If condensation is present at the end of the 65°C incubation, briefly centrifuge the plate for 1–2 minutes at 1,500 × g.
2. Add 200 µL of Multi-Sample DNA Lysis Buffer to each sample.
3. Seal the plate with a clear adhesive film, then shake for 5 minutes at 900–950 rpm.
4. Transfer lysates to the wells of a new deep-well plate and discard the plate containing the buccal swabs.
5. Add 240 µL of isopropanol to each sample, seal the plate, then shake for 5 minutes at 900–950 rpm.
6. Vortex DNA Binding Bead Mix at moderate speed to ensure thorough mixing, add 40 µL to each sample, then proceed immediately to DNA isolation (next section).
If you see that the beads in the DNA Binding Bead Mix are settling, vortex the mix again briefly before continuing to pipette.

Process samples on the instrument

1. Select the program on the instrument.
 - KingFisher™ Flex Magnetic Particle Processor: **A25597_Blood_Buccal**
 - MagMAX™ Express-96 Magnetic Particle Processor: **4413021 DW blood**
2. Start the run, remove the temporary paraffin plate seals (if present), then load the prepared processing plates in their positions when prompted by the instrument.
3. Load the PK plate (containing lysate, isopropanol, and DNA Binding Bead Mix) at position 1 when prompted by the instrument.
4. When prompted by the instrument (approximately 28–30 minutes after initial start):
 - a. Remove the Elution Plate from the instrument.

- b. Add 50 µL of DNA Elution Buffer 2 to each sample well.

IMPORTANT! Add DNA Elution Buffer 2 immediately after the prompt, to prevent excessive drying of any beads that are still captured on the Tip Comb.

- c. Load the Elution Plate back onto the instrument, and press **Start**.

5. At the end of the run (approximately 30–35 minutes after initial start), remove the Elution Plate from the instrument and seal immediately with a new clear adhesive film.
- (Optional) Eluates can be transferred to a new storage plate after collection.
 - If precipitated DNA is visible, pipet up and down 5–10 times before sealing the plate, to ensure complete resuspension.
 - If you see excessive bead residue in the wells, place the Elution Plate on the Magnetic Stand-96 to capture any residue prior to downstream use of the DNA.

IMPORTANT! Do not allow the purified samples to sit uncovered at room temperature for more than 10 minutes, to prevent evaporation and contamination.

The purified samples are ready for immediate use. Alternatively, store the covered Elution Plate:

- At 2–6°C for up to 24 hours.
- At –20°C or –80°C for long-term storage.

Isolate DNA from whole blood

This section contains brief procedures. For detailed information, refer to *MagMAX™ DNA Multi-Sample Ultra Kit (whole blood) User Guide* (Pub. No. MAN0010294).

Sample collection and storage

- **Sample collection:** Collect blood samples using proper venipuncture collection and handling procedures in EDTA or sodium citrate anticoagulant tubes. Invert the tube to ensure thorough mixing.

Note: Heparin is not recommended as an anti-coagulant since it can cause inhibition of PCR.

- (Optional) **Sample storage:** Store samples between –20°C and –80°C. We recommend storing samples in smaller volumes to prevent multiple freeze/thaw cycles.

Guidelines for whole blood preparation

- If the whole blood is frozen prior to use, thaw the sample at 25–37°C in a water bath until it is completely liquid, then place on ice until needed.
- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- When mixing samples by pipetting up and down, avoid creating bubbles.
- Cover the plate during the incubation and shaking steps to prevent spill-over and cross-contamination. The same MicroAmp™ Clear Adhesive Film can be used throughout the procedure, unless it becomes contaminated.

- If you use a plate shaker other than the recommended shaker, verify that:
 - The plate fits securely on your plate shaker.
 - The recommended speeds are compatible with your plate shaker. Ideal shaker speeds allow for thorough mixing without splashing.
- Per-plate volumes for reagent mixes are sufficient for one plate plus overage. To calculate volumes for other sample numbers, refer to the per-well volume and add 5% overage.
- If the DNA yield is lower than expected, extend the Proteinase K digestion to 45 minutes.

Before first use of the kit

Prepare the Wash Solutions from the concentrates:

- Add 25 mL of isopropanol to Wash Solution 1 Concentrate, mix, and store at room temperature.
- Add 132 mL of ethanol to Wash Solution 2 Concentrate, mix, and store at room temperature.

Before each use of the kit

Preheat the incubator to 65°C.

Digest the samples with Proteinase K

Ensure that the incubator is preheated to 65°C.

1. Prepare sufficient PK Mix according to the following table, then invert several times to thoroughly mix components.

IMPORTANT! Prepare the PK Mix just before use. Do not place the PK Buffer or the PK Mix on ice, to avoid precipitation.

Component	Volume per well	Volume per plate
Proteinase K	8 µL	800 µL
PK Buffer	192 µL	19.2 mL
Total PK Mix	200 µL	20 mL

2. Add 200 µL of PK Mix to each sample well of a deep-well plate (PK Plate).
3. Transfer 50 µL of whole blood to the appropriate well containing PK Mix.

IMPORTANT! Invert the tube containing the blood sample before pipetting to ensure homogenous mixing.

4. Seal the plate with a clear adhesive film, then shake the sealed plate for 5 minutes at 900–950 rpm.
5. Incubate for 20–45 minutes at 65°C.

IMPORTANT! Arrange plates in the incubator to allow adequate flow around the plate wells, to ensure that samples quickly reach and maintain the incubation temperature.

Set up the processing plates

1. While the samples are incubating at 65°C, set up the Wash, Elution, and Tip Comb Plates outside the instrument as described in the following table.

Plate ID	Plate position ^[1]	Plate type	Reagent	Volume per well
Wash Plate 1	2	Deep Well	Wash Solution 1	150 µL
Wash Plate 2	3	Deep Well	Wash Solution 2	150 µL
Wash Plate 3	4	Deep Well	Wash Solution 2	150 µL
Elution Plate ^[2]	5	Standard	DNA Elution Buffer 1	50 µL
Tip Comb	6	Deep Well	Place a tip comb in the plate.	

^[1] Position on the instrument

^[2] The instrument prompts the user to add DNA Elution Buffer 2 to the Elution Plate, after incubation with DNA Elution Buffer 1.

2. (Optional) To prevent evaporation and contamination, cover the prepared processing plates with paraffin film until they are loaded into the instrument.

Add Multi-Sample DNA Lysis Buffer, isopropanol, and Bead/RNase A Mix

1. (Optional) If condensation is present at the end of the 65°C incubation, briefly centrifuge the plate for 1–2 minutes at 1,500 × g.
2. Add 200 µL of Multi-Sample DNA Lysis Buffer to each sample.
3. Seal the PK plate with a clear adhesive film, then shake for 5 minutes at 900–950 rpm.
4. Prepare Bead/RNase A Mix according to the following table.

IMPORTANT! Prepare the Bead/RNase A Mix no more than 20 minutes before use. Prolonged storage at room temperature can reduce its efficiency.

Vortex the DNA Binding Beads at moderate speed to form a uniform suspension before preparing the Bead/RNase A Mix.

Component	Volume per well	Volume per plate
DNA Binding Beads	16 µL	1.6 mL
RNase A	5 µL	500 µL
Nuclease-free Water	19 µL	1.9 mL
Total Bead/RNase A Mix	40 µL	4.0 mL

5. Vortex the Bead/RNase A Mix at moderate speed to ensure thorough mixing, then add 40 µL to each sample and pipet up and down 5 times using a multi-channel micropipettor.
If you see that the beads in the Bead/RNase A Mix are settling, vortex the mix again briefly before continuing to pipette.
6. Seal the PK plate with the clear adhesive film, then shake for 5 minutes at 900–950 rpm.
7. Add 240 µL of isopropanol to each sample, then proceed immediately to DNA isolation (next section).

Process samples on the instrument

1. Select the program on the instrument.
 - KingFisher™ Flex Magnetic Particle Processor: **A25597_Blood_Buccal**
 - MagMAX™ Express-96 Magnetic Particle Processor: **4413021 DW blood**
2. Start the run, remove the temporary paraffin plate seals (if present), then load the prepared processing plates in their positions when prompted by the instrument.
3. Load the PK plate (containing lysate, isopropanol, and Bead/RNase A Mix) at position 1 when prompted by the instrument.
4. When prompted by the instrument (approximately 28–30 minutes after initial start):
 - a. Remove the Elution Plate from the instrument.
 - b. Add 50 µL of DNA Elution Buffer 2 to each sample well.

IMPORTANT! Add DNA Elution Buffer 2 immediately after the prompt, to prevent excessive drying of any beads that are still captured on the Tip Comb.

 - c. Load the Elution Plate back onto the instrument, and press **Start**.
5. At the end of the run (approximately 30–35 minutes after initial start), remove the Elution Plate from the instrument and seal immediately with a new clear adhesive film.
 - (*Optional*) Eluates can be transferred to a new storage plate after collection.
 - If precipitated DNA is visible, pipet up and down 5–10 times before sealing the plate, to ensure complete resuspension.
 - If you see excessive bead residue in the wells, place the Elution Plate on the Magnetic Stand-96 to capture any residue prior to downstream use of the DNA.

IMPORTANT! Do not allow the purified samples to sit uncovered at room temperature for more than 10 minutes, to prevent evaporation and contamination.

The purified samples are ready for immediate use. Alternatively, store the covered Elution Plate:

- At 2–6°C for up to 24 hours.
- At –20°C or –80°C for long-term storage.

Prepare a DNA stock solution for OpenArray™ experiments

The recommended input for OpenArray™ plates is 825 pg of DNA per 33-nL reaction.

1. Quantify DNA by any of the following methods. Before quantifying, mix the samples well to ensure sample homogeneity, especially if samples have been stored.
 - **RNase P C_t value method** (recommended)—See Appendix C, “RNase P quantification for genotyping experiments”.
 - **Fluorometric analysis**—Use a Qubit™ dsDNA BR or HS Assay Kit.
2. Prepare a 50 ng/μL DNA stock solution to use in the PCR setup.
Samples with low concentration (<10 ng/μL) and/or low quality may require preamplification. See Appendix B, “Preamplification of low-concentration gDNA”.

Normalize DNA samples for copy number analysis

The required input for each 10-μL PCR is 10 ng of DNA.

Run each sample in quadruplicate for each copy number assay.

1. Dilute a portion of each gDNA sample to 5 ng/μL. Prepare sufficient DNA for the required number of reactions.
 $(\# \text{ of assays}) \times (\text{Vol. of } 5 \text{ ng}/\mu\text{L DNA per } 10 \mu\text{L reaction}) \times (\# \text{ of replicates}) \times 120\%$
(for dead volume)
For example, if you are running 5 copy number assays, prepare at least:
 $(5 \text{ Assays}) \times (2 \mu\text{L}) \times (4 \text{ replicates}) \times 120\%$
 $= 48 \mu\text{L}$ of each sample at a concentration of 5 ng/μL
2. Store the diluted samples at 4°C for immediate use or at -25°C to -15°C for long-term storage.



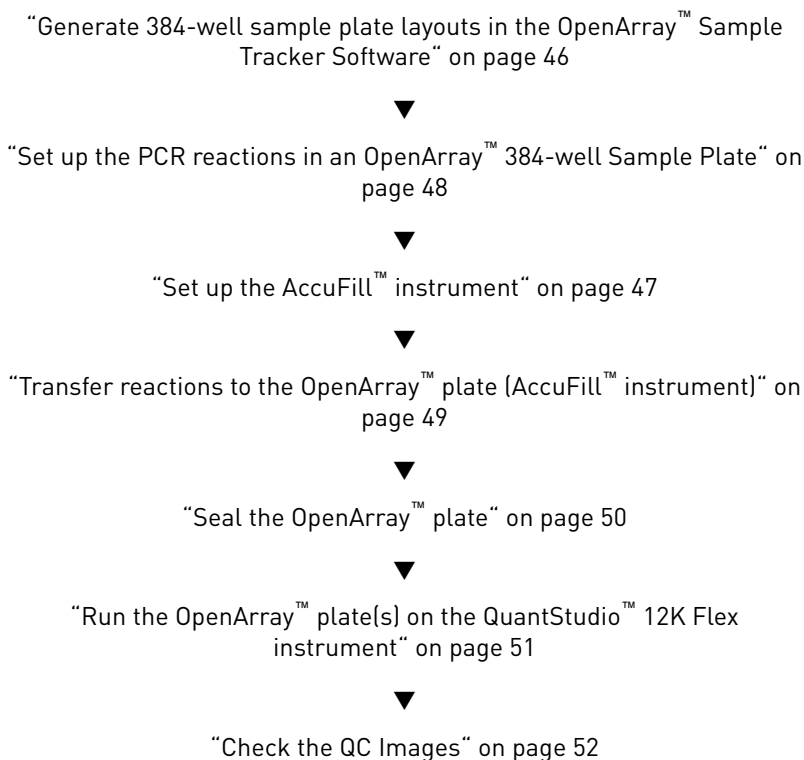
Prepare and run OpenArray™ PGx SNP genotyping experiments

- Workflow 43
- Materials required: OpenArray™ plate workflow 43
- Recommended: Run OpenArray™ SNP genotyping experiments in real-time mode 44
- Generate 384-well sample plate layouts in the OpenArray™ Sample Tracker Software 46
- Set up the AccuFill™ instrument 47
- Set up the PCR reactions in an OpenArray™ 384-well Sample Plate 48
- Transfer reactions to the OpenArray™ plate (AccuFill™ instrument) 49
- Seal the OpenArray™ plate 50
- Run the OpenArray™ plate(s) on the QuantStudio™ 12K Flex instrument 51
- Check the QC Images 52
- One-time procedures 53
- Download SPF Files 54

This chapter contains brief procedures. For detailed procedures, refer to:

Title	Pub. No.
<i>QuantStudio™ 12K Flex Real-Time PCR System: OpenArray™ Experiments User Guide</i>	4470935
<i>OpenArray™ Sample Tracker Software Quick Reference</i>	4460657
<i>OpenArray™ AccuFill™ System User Guide</i>	4456986
<i>OpenArray™ SNP Genotyping Experiments</i>	MAN0014351
<i>TaqMan® OpenArray™ Genotyping Troubleshooting Guide</i>	MAN0011115
<i>TaqMan® Genotyper Software Getting Started Guide</i>	4448637

Workflow



Materials required: OpenArray™ plate workflow

Unless otherwise indicated, all materials are available through **thermofisher.com**.
MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

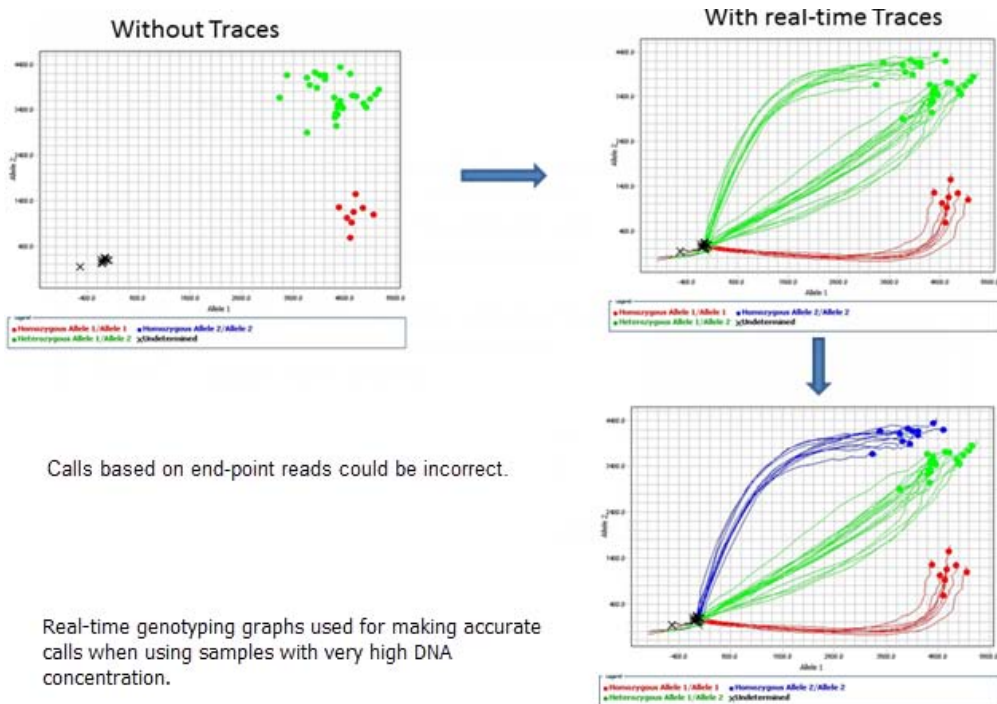
Item	Source
Genomic DNA, 50 ng/μL	From Chapter 3, “Isolate DNA using the MagMAX™ DNA Multi-Sample Ultra Kit”
OpenArray™ 384-well Sample Plate	4482221
OpenArray™ plates with PGx assays	Custom ordered
TaqMan® OpenArray™ Genotyping Master Mix	4404846
Biomek Seal and Sample Foil Lids (for pre-plating step)	Beckman Coulter 538619
OpenArray™ AccuFill™ System Tips	4458107
QuantStudio™ 12K Flex OpenArray™ Accessories Kit (contains the items needed to assemble up to 10 plates: 12 lids and plugs, 12 immersion fluid syringes, and 2 carriers)	4469576
OpenArray™ Sample Tracker Software	Download from thermofisher.com .

Item	Source
QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0	A24945
QuantStudio™ 12K Flex instrument with OpenArray™ block (AccuFill™ System)	Contact your local customer service representative.

Recommended: Run OpenArray™ SNP genotyping experiments in real-time mode

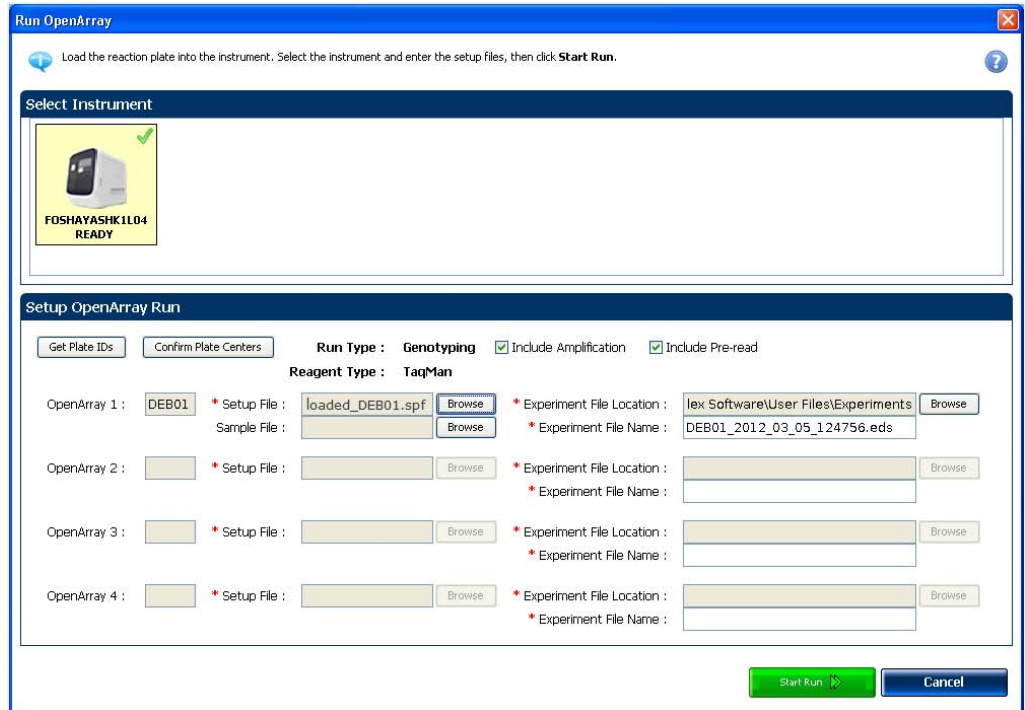
We recommend running all OpenArray™ plates on a QuantStudio™ instrument in real-time experiment mode. This recommendation allows observation of genotyping data over time to evaluate the accuracy of genotype calls by observing the location of a given sample relative to others throughout all cycles.

The following example shows how calls based on end-point reads could be incorrect when the DNA concentration is higher than the recommended amount.



Note: Generally, a C_T value of ~25 indicates that the sample input is approximately 250 haploid copies, which is the recommended input amount per 33-nL reaction for OpenArray™ genotyping plates. This corresponds to 825 pg of DNA per PCR reaction using a stock solution of 50 ng/μL.

The default run type for genotyping experiments is real-time mode, indicated by the selection of **Include Amplification** in the **Setup OpenArray Run** window. The default settings also include the selection of **Include Pre-read**; this setting is strongly recommended to correct for background fluorescence on the plate.



For additional information about genotyping experiments, see the *QuantStudio™ 12K Flex Real-Time PCR System: OpenArray™ Experiments User Guide* (Pub. No. 4470935).

Generate 384-well sample plate layouts in the OpenArray™ Sample Tracker Software

The software maps samples from the 96-well layout used for DNA isolation to a 384-well sample plate layout as *.csv files that are used by OpenArray™ AccuFill™ software.

See “One-time procedures” on page 53 to:


- Set up optimized folder locations and software preferences before performing this procedure for the first time.
 - Download the *.spf file(s) for the OpenArray™ plate(s) before starting.
1. Generate the 96-well sample plate *.csv file using the OA_Genotyping_CalcSheet or 96-Well Sample Plate 1.csv template, then save it to the Sample Tracker 96-well Input folder.
The 96-Well Sample Plate 1.csv file is provided in the AccuFill™ software installation. Enter or copy the sample names in 96-Well Sample Plate 1.csv, then **Save as** a new *.csv-format file.
The OA_Genotyping_CalcSheet automatically generates the sample *.csv file.
 2. In the Sample Tracker Software **Properties** screen, select **Genotyping** for **Experiment Type**, then select the appropriate settings for **OpenArray™ Plate** and **Pipettor**.
 3. In the **Samples** screen, click  **Import**, then select and import the sample *.csv file.
 4. In the **Sample Mapping** screen, confirm that the samples for a single OpenArray™ plate are assigned to one color.
If necessary, correct the **OpenArray™ Plate** and **Pipettor** settings in the **Properties** screen.
 5. In the **Sample Mapping** screen, click the **384-Well Plate** tab, then click **Export ▶ Export *.csv**.
 6. Select **384-Well Plate (for AccuFill)**, then save the exported file.

Plate layouts for the 384-well sample plates are saved to individual *.csv files in the Sample Tracker 384-well CSV Files folder.

Set up the AccuFill™ instrument

IMPORTANT! Before proceeding, check the tip expiration date (shown on the outer box that contains the trays of tips). Do not use tips that exceed the expiration date.

1. In the OpenArray™ AccuFill™ software, click **Setup and Load**.
2. In the Setup Load Information window, verify that the **Use Sample Integration** checkbox is selected.

Setup Load Information

Loading information

Specify the type of load you want to perform. Enter Plate and OpenArray® Plate barcodes by scanning them, typing them, or selecting the associated file.

Use Sample Integration Samples Per Subarray 1

Sample Plate Plate Holder Position 1

Plate Holder Position 2

Plate Holder Position 3

Plate Holder Position 4

Select Samples to Load

Click the section on the sample plate from which the first sample will be extracted. The image on the right shows how the sample will be applied to the OpenArray Plates.

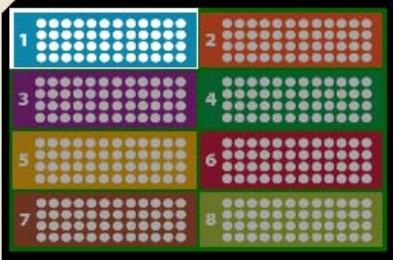


Plate Holder Position 1

Plate Holder Position 2

Plate Holder Position 3

Plate Holder Position 4

Sample section(s) already used: 1 on 2/1/2012

3. Click **Browse** to the right of **Sample Plate**, then select the 384-well sample plate *.csv file that was generated with the OpenArray™ Sample Tracker Software.
4. Click **Browse** to the right of the plate holder position corresponding to the OpenArray™ of interest, then select the *.spf file corresponding to the desired OpenArray™ plate.

5. Click the corresponding 4 × 12 area of the 384-well plate, then click **Next** to open the **Setup Deck** window.
6. Ensure that:
 - Tip boxes are loaded in the AccuFill™ instrument in the displayed configuration.
 - Lids are removed from the tip boxes.
 - The waste bin in the instrument is emptied.
7. In the **Setup Deck** window, select:
 - **The tips are configured as shown above**
 - **The Waste Bin is empty**

Set up the PCR reactions in an OpenArray™ 384-well Sample Plate

For best results, dilute DNA samples to 50 ng/μL before setting up the PCR reactions.

IMPORTANT! The 4 × 12 area(s) of the 384-well plate being filled must match the area(s) designated in the OpenArray™ Sample Tracker Software for that set of samples.

1. Remove the OpenArray™ plate from the freezer; allow it to come to room temperature in its unopened sleeve (~15 minutes).
 The OpenArray™ plate must be completely thawed before transferring reactions to it from the 384-well sample plate.
2. Mix the 2X Master Mix gently. Do not invert the bottle.
3. Vortex the Assay Working Stock, then centrifuge briefly.
4. Following the plate layout designated in the OpenArray™ Sample Tracker Software, add master mix, then UltraPure™ DNase/RNase-Free Distilled Water (NTCs) or DNA samples, to an OpenArray™ 384-well Sample Plate.

Note: Include 2 NTCs on each plate.

Component	OpenArray™ Genotyping Plate Format		
	16	32	64 +
TaqMan® OpenArray™ Genotyping Master Mix	1.5 μL	2.0 μL	2.5 μL
One of the following: <ul style="list-style-type: none"> • UltraPure™ DNase/RNase-Free Distilled Water (NTCs) • DNA sample 	1.5 μL	2.0 μL	2.5 μL
Total reaction volume	3.0 μL	4.0 μL	5.0 μL

5. Seal the plate with an aluminum foil seal, remove the foil flap, mark the edges of the filled 4 × 12 area with a pen, then score the foil along those lines.
Do not remove the foil from the scored area at this time.
6. Centrifuge the plate at 1,000 rpm for 1 minute.

If you make a sample layout error:

- Before the AccuFill™ procedure – Repeat “Generate 384-well sample plate layouts in the OpenArray™ Sample Tracker Software” on page 46 with a corrected sample.csv file.
- After the AccuFill™ procedure – See “Recover from layout errors in the 384-well sample plate” on page 80.

Transfer reactions to the OpenArray™ plate (AccuFill™ instrument)

Ensure that the OpenArray™ plate is thoroughly thawed before starting this procedure.

1. Prepare the items needed to seal the OpenArray™ plate (next section).
Note: The OpenArray™ plate must be sealed promptly after being loaded with the reactions (this section).
 - a. Ensure that the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0 is ready.
 - b. Gather and remove from packaging an OpenArray™ lid, plug, syringe with OpenArray™ Immersion Fluid, and syringe tip.
 - c. Attach the syringe tip to the syringe and carefully push some of the fluid through the tip to remove air bubbles, then lay the syringe aside.
2. Remove the OpenArray™ plate from its sleeve and place it in the plate holder of the AccuFill™ instrument.
Ensure that the bar code on the OpenArray™ plate is facing left and the serial number is facing right.
3. Using forceps, peel the foil from the filled area of the OpenArray™ 384-well Sample Plate.
4. Close the instrument door.
5. In the AccuFill™ software **Setup Deck** window, select the following confirmations, then click **Load**.
 - **The OpenArray Plate is in the Plate Holder**
 - **Remove foil from the highlighted section of the Sample Plate**
6. As soon as the Remove OpenArray Plate window appears, open the instrument door, then remove the loaded OpenArray™ plate.

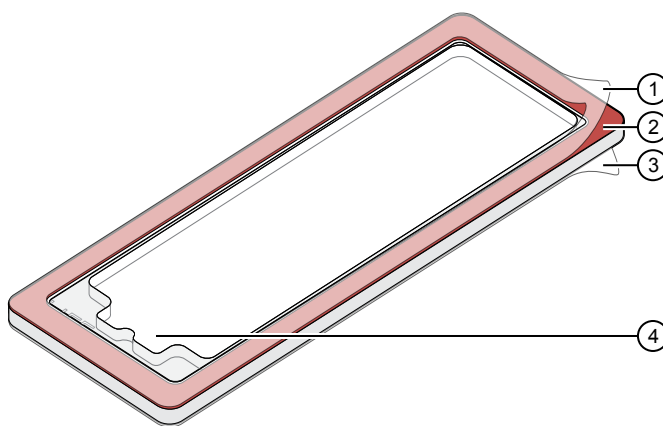
7. Proceed immediately to seal the OpenArray™ plate (next section).

Note: For best results, seal the OpenArray™ plate within 90 seconds of completion of loading, to prevent evaporation.

Seal the OpenArray™ plate

IMPORTANT! Handle the OpenArray™ plate and case only by the edges throughout this procedure.

1. Place the filled OpenArray™ plate in the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0.
Ensure that the bar code is facing left and the serial number is facing right.
2. Remove the clear plastic sheets from the top and the bottom of the lid, remove the red protective film around the edge of the OpenArray™ lid, then seat the lid on the OpenArray™ case in the plate press.



- ① Protective film (remove)
- ② Adhesive
- ③ Protective film (remove)
- ④ Notched end (align with serial number)

3. Engage the press mechanism until the green flashing light changes to a steady green light (~20 seconds).
4. Disengage the press, then remove the OpenArray™ case.

5. While holding the OpenArray™ case by the edges, insert the prepared syringe tip into the port in the case, then carefully inject immersion fluid until the case is filled.

Note: Minimize creation of air bubbles when you dispense the fluid; one small air bubble in the case is acceptable.






The syringe tip must be in front of the array when filling the case with immersion fluid.

6. While holding the case vertically, remove the syringe tip, insert the screw end of the OpenArray™ plug into the port and rotate clockwise until the black handle breaks off.

IMPORTANT! To avoid leaking of immersion fluid, hold the case vertically and rotate the plug slowly.

7. Clean the case with a laboratory wipe that has been thoroughly sprayed with ethanol, then dry the case with a clean laboratory wipe.


Run the OpenArray™ plate(s) on the QuantStudio™ 12K Flex instrument

1. On the instrument touchscreen, touch  to extend the loading arm, and place the OpenArray™ plates on the plate adapter.
Ensure that the plate barcode and serial number are facing the front of the instrument.
2. Touch  to retract the loading arm.
3. In the  Home screen of the QuantStudio™ 12K Flex Software, select **Run** ▶ **OpenArray**.
4. In the **Select Instrument** pane, select your QuantStudio™ instrument.

5. Click **Get Plate IDs** to import the barcode(s) of the OpenArray™ plate(s).
Once the OpenArray™ serial numbers appear, the loaded *.spf files corresponding to each plate should appear in the **Setup File** field.
If not, click **Browse**, then select the correct loaded *.spf file from the Loaded SPF folder.
6. (Optional) Click **Browse** to change the QuantStudio™ **Experiment File Location**.
7. (Optional) Change the software-determined **Experiment File Name**.
8. Click **Start Run**.
Note: The instrument pauses at 41 or 42 seconds prior to the end of the run. Wait for the system to complete the run before opening the *.eds file.
9. Transfer the *.eds file from the instrument to an accessible location for analysis in TaqMan® Genotyper Software.

Check the QC Images

Check the QC images before analysis in TaqMan® Genotyper Software. For additional information, see *TaqMan® OpenArray™ Genotyping Troubleshooting Guide* (Pub. No. MAN0011115).

1. In the QuantStudio™ 12K Flex Software  **Export** screen:
 - a. Click **Browse** to create a uniquely-named folder for the QC images export.
 - b. Click **Export QC Images** (bottom of screen).

IMPORTANT! Create a new folder for images each time; exporting a second run to the same folder overwrites the images.

2. View the following ROX™ image to check for loading quality issues:
 - POST-READ_CHANNEL_4.tiff
3. Check for leaks or other displaced sample issues.
 - a. View the following spotfinding images:
 - s03_c001_t03_p0001_m2_x3_e1_cp#_spotfind.tiff
 - s04_c001_t02_p0001_m2_x3_e1_cp#_spotfind.tiff

Note: The “cp#” in the image file name refers to the array position (1–4) within the instrument.
 - b. If a problem is found, view the following pre-run spotfinding image to determine if the issue existed even before cycling (this is useful for troubleshooting):
 - s00_c001_t01_p0001_m2_x3_e1_cp#_spotfind.tiff

4. View the following FAM™ and VIC™ images to check for any fluorescent abnormalities and to confirm any problem seen in the spotfinding images:
 - STAGE4_CYCLE1_CHANNEL_1.tiff
 - STAGE4_CYCLE1_CHANNEL_2.tiff
5. Note any abnormalities found, as well as all other potentially relevant information related to the setup of the run.

One-time procedures

Set up default folders and software preferences

This procedure simplifies the file locations used in the OpenArray™ AccuFill™ instrument software.

Set up the default file locations and preferences before using the OpenArray™ AccuFill™ system for the first time. You must be logged in as an administrator.

1. Create the following four folders in a convenient location on the same computer drive as the AccuFill™ software:
 - SPF Files
 - Sample Tracker 96-well Input
 - Sample Tracker 384-well CSV Files
 - Loaded SPF Files
2. (*Optional*; not required if using the OA_Genotyping_CalcSheet) Navigate to <drive>:\Program files (x86)\AppliedBiosystems\OpenArray Sample Tracker\Examples, copy the 96-Well Sample Plate 1.csv file, then paste it in the Sample Tracker 96-well Input folder.
3. In OpenArray™ Sample Tracker Software, select **View ▶ Preferences**, then enter the following preferences:

Field	Selection
Experiment type	Genotyping
OpenArray™ Plate	Select the OpenArray™ format that will be run most often, for example, Genotyping – 64
Pipettor	Fixed or Adjustable
Import Data Directory	Sample Tracker 96-well Input folder
Export Data Directory	Sample Tracker 384-well CSV Files folder

4. In the AccuFill™ software, select **Instrument ▶ Edit Preferences**, then:
 - a. Select **Require Sample Integration**.

- b. Select the indicated folders.

For this AccuFill™ folder...	Select this folder...	Folder contents
OpenArray™ Plate File Input Folder	SPF Files	*.spf files for your OpenArray™ plates; contain assay name and location
Sample Plate File Folder	Sample Tracker 384-well CSV Files	*.csv 384-well sample plate layout files
Loaded OpenArray™ Plate File Folder	Loaded SPF Files	Integrated *.spf files that are generated during processing with the AccuFill™ software.

5. In the QuantStudio™ 12K Flex instrument software, select **Tools ▶ Preferences ▶ OpenArray**, then select the **Loaded SPF Files** folder for the software **Setup Folder**.

Note: If the instrument software is not on same computer as the AccuFill™ software, transfer the loaded *.spf files to the computer running the instrument software.

Download SPF Files

Set up the optimized folder locations and software preferences before downloading *.spf files. See “Set up default folders and software preferences” on page 53.

For custom OpenArray™ plates, you need the **Lot#** and the **Serial#** from the packaging of each OpenArray™ plate.

1. At www.thermofisher.com/OA-platefiles, select **TaqMan® OpenArray™ Custom Gene Expression/Genotyping Plates** in the **Select Your Product** dropdown list.
2. Select a download option:
 - **I want to download all available SPF & AIF files**
 - **I want to download a specific SPF file**
3. Enter the **Lot#** and the **Serial#**, then click **Submit**.
Note: The **Serial#** is case-sensitive.
4. Save the *.spf files to the desktop **SPF Files** folder.

Note: Do not create sub-folders in the **SPF Files** folder. The software cannot access sub-folders.

Analyze OpenArray™ PGx SNP genotyping experiments

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■ Analyze data in QuantStudio™ 12K Flex or Thermo Fisher Cloud software ..	57
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This chapter contains brief procedures. For detailed procedures, refer to:

Title	Pub. No.
<i>QuantStudio™ 12K Flex Real-Time PCR System: OpenArray™ Experiments User Guide</i>	4470935
<i>OpenArray™ Sample Tracker Software Quick Reference</i>	4460657
<i>OpenArray™ AccuFill™ System User Guide</i>	4456986
<i>OpenArray™ SNP Genotyping Experiments</i>	MAN0014351
<i>TaqMan® OpenArray™ Genotyping Troubleshooting Guide</i>	MAN0011115
<i>TaqMan® Genotyper Software Getting Started Guide</i>	4448637

Before you begin analysis (each time)

Transfer files from QuantStudio™ 12K Flex Software with a network connection

1. In the Home screen of the QuantStudio™ 12K Flex Software, click **Instrument Console**.
2. In the Instrument Console, select the desired QuantStudio™ 12K Flex Instrument from the list of instruments on the network, then click **Add to My Instruments**.
3. After the QuantStudio™ 12K Flex Instrument is added to your list, select it, then click **Manage Instrument**.
4. In the Instrument Manager, click **Manage Files**, then click **File Manager**.
5. In the **File Manager** screen, transfer the file(s) from the QuantStudio™ 12K Flex Instrument:
 - a. In the **Folders** field, select the folder that contains the files that you want to download.
 - b. In the **Experiments** field, select the files to download. To select multiple files, Ctrl-click or Shift-click files in the list.
 - c. When you have selected the files that you want to download, click **Download**.
 - d. In the **Send experiment to instrument** dialog box, select the folder to which you want to download the selected file(s), then click **Open**.

For more details on file transfers please refer to the networking chapter in the *QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide* (Pub. No. 4470689).

Transfer files from QuantStudio™ 12K Flex Software without a network connection

Download and save files in one of the following ways:

- Connected computer
 - Browse for the folder containing the run.
 - Select run names and download the file to storage media.
- Instrument only
 - Insert a USB storage device into one of the USB ports in the front panel.
 - Use the touchscreen and press **Collect Results**.
 - Select run names and press **Save to USB**.

Analysis options for genotyping data

Analyze allelic discrimination plots for genotyping data using any of these tools:

Software	Features
QuantStudio™ 12K Flex software	<ul style="list-style-type: none"> • Desktop software • View real-time trace data to aid genotype calling
Thermo Fisher Cloud Genotyping Application	<ul style="list-style-type: none"> • Cloud software • Create studies • Overlay data from multiple plates • View real-time trace data to aid genotype calling
TaqMan® Genotyper Software	<ul style="list-style-type: none"> • Desktop software • Create studies • Overlay data from multiple plates

Analyze data in QuantStudio™ 12K Flex or Thermo Fisher Cloud software

For non-standard assays, review the data for both assays together to ensure accurate genotyping results.

To use the QuantStudio™ 12K Flex software or related instrument software:

1. In the analysis settings, select **Analyze Real-Time Rn – Median (Rna to Rnb)**. This setting will normalize for any run and system noise to improve data accuracy.
2. Select **Analysis ▶ Allelic Discrimination Plot**.

To use the Thermo Fisher Cloud GT Analysis module:

1. Go to **thermofisher.com/cloud**.
2. Create a project and import one or more OpenArray™ experiment files (.eds).
3. Access the Genotyping (GT) application.
4. Analyze data.

Analyze data with TaqMan® Genotyper Software

Before you begin TaqMan® Genotyper Software analysis

Create a study template

If using a reference panel, generate the reference panel before creating the study template, so that the reference panel can be imported into the template.

If necessary, first download the Assay Information File (AIF) from <http://www.thermofisher.com/OA-platefiles>.

1. In the TaqMan® Genotyper Software **Home** screen, click **Create Study**.
2. In the **Workflow Menu** pane, select **Setup ▶ Properties**, then enter the study properties.
 - For **Instrument Type**, select **QuantStudio™ 12K Flex Real-Time PCR System**.
 - For **Experiment Type**, select **Real-time**.
3. In the **Workflow Menu** pane, select **Setup ▶ Assays**.
4. Click **Import**, then select and import the Assay Information File (AIF).
5. (*Optional*) Import the reference panel:
 - a. In the **Workflow Menu** pane, select **Setup ▶ References**.
 - b. Click **Import**, then select and import the reference panel file of interest.
6. Select **File ▶ Generate Template**, enter a template name (.edt), then save the template in the desired location.

(*Optional*) Generate a reference panel

A reference panel consists of high-quality data for all the assays in the study. The data can be chosen from multiple OpenArray™ plates.

For best results, generate a new reference panel for each lot of OpenArray™ plates.

If necessary, first create a study containing data that will be used as reference samples. For the study properties options, set **QuantStudio™ 12K Flex Real-Time PCR System** for **Instrument Type**, and **Real-time** for **Experiment Type**.

1. In the TaqMan® Genotyper Software, open the study that contains data points to be used as reference samples.
2. In the **Workflow Menu** pane, select **Analysis ▶ Results**.
3. Click-drag in the scatter plot to select one or more data points.
Select data points for each homozygous genotype (FAM™ dye-labeled or VIC™ dye-labeled) and the heterozygous genotype.
The non-selected data points will fade.
4. Right-click in the scatter plot, then select **Tag for Ref Panel**.
5. Repeat the selection and tag steps for additional data points.

6. Confirm that the correct samples have been tagged as reference samples in the **Results** table.
In the **Results** tab, **Reference Sample** must be selected in the **View** dropdown list to view the tags.
7. Repeat the data point selection, tagging, and confirmation steps for each SNP assay of interest.
8. Select **File** ▶ **Save**.
9. Select **File** ▶ **Generate Reference Panel**, enter a name for the panel, then save the file (.lap) to the desired location.

(Optional) Add samples to an existing reference panel

During analysis in TaqMan® Genotyper Software, you can add samples to an existing reference panel.

The reference panel must already be imported into the study containing samples that will be added to the reference panel.

1. In the scatter plot of the study, select data points for the samples of interest, right-click in the plot, then select **Tag for Ref Panel**.
2. Select **File** ▶ **Save**.
3. Select **File** ▶ **Generate Reference Panel**, enter a name for the panel, then save the file (.lap) to the desired location.

Analyze results in TaqMan® Genotyper Software

1. In TaqMan® Genotyper Software, click **Create Study from Template**, then select and open the desired template (.lat).
Note: If you have not created a template, see “Create a study template” on page 58.
2. In the **Workflow Menu** pane, select **Setup** ▶ **Experiments**.
3. Click **Import**, then select and import one or more OpenArray™ experiment files (.eds).
4. Click **Analyze**.
5. Inspect the call data for the SNP assay in the scatter plot.
Refer to the *TaqMan® Genotyper Software Getting Started Guide* (Pub. No. 4448637) for detailed instructions.
6. *(Optional)* To manually assign the genotype, select the sample(s), right-click, then select the genotype.
7. Select **Inspected** next to the assay, then click **Save**.
8. Repeat the inspection for each SNP assay.
9. In the **Workflow Menu** pane, select **Export** ▶ **Analysis Data**.

10. In the **Export Study Properties** pane:
 - a. Select **Analysis Results ▶ Advanced**.
 - b. Select **One File**.
 - c. Enter the filename and location, and select **(* .txt)**.
11. Click **Export preview**, then click **Start Export** in the new window.

Review call rates and other QC parameters

Select the appropriate tab to review call rates and other QC parameters in TaqMan® Genotyper Software.

To...	Do this...
Review sample call rates and QC data	<ol style="list-style-type: none"> 1. Select Analysis ▶ Quality Control. 2. Select the Samples tab to review the sample call rates and other QC parameters. Samples with low call rates are an indication of poor sample quality or concentration and it is advisable to omit those samples from the study. 3. <i>[Optional]</i> To omit samples with poor quality or concentration, right click the sample name, select Omit from the dropdown menu, then reanalyze the data.
Review plate call rates and QC data	<ol style="list-style-type: none"> 1. Select Analysis ▶ Quality Control. 2. Review call rates and QC data in the Experiments tab.
Review assay call rates and QC data	<ol style="list-style-type: none"> 1. Select Analysis ▶ Quality Control. 2. Review call rates and QC data in the Assay tab.

For detailed instructions, see the *TaqMan® Genotyper Software Getting Started Guide* (Pub. No. 4448637).

Analysis guidelines: sex chromosome targets

In most cases, **Use Hardy-Weinberg for Analysis** is selected. X and Y chromosome-specific SNP targets do not follow a Hardy-Weinberg distribution.

To improve the accuracy of genotype calls for SNP targets on the X and Y chromosomes:

- Deselect **Use Hardy-Weinberg for Analysis**
- Select **Disallow in Males** from the **Heterozygote** drop-down list

Note: If you select **Disallow in Males** for other types of targets, the genotype call accuracy may be negatively impacted.

SNP targets located in the PAR region of the X and Y chromosomes behave like autosomal SNP targets and males can be heterozygotes. The gender assay (C_990000001_10) target X- and Y- chromosome specific sequences in the amelogenin gene: males run as heterozygotes and females run as VIC™ homozygotes. Do not select **Disallow in Males** with these assay types.

Analysis guidelines: DME genotyping assays for genes in copy number variation regions

Some TaqMan® DME Assays target polymorphisms in genes that exhibit copy number variation including CYP2D6, CYP2A6, CYP2E1, GSTM1, GSTT1, and SULT1A1. Copy number variation analysis must be done in addition to genotyping with DME assays. The frequency of the CYP2D6, CYP2A6, CYP2E1, and SULT1A1 gene deletions are low, and samples that carry no copies of these genes will be rare. However, the frequency of deletion of GSTM1 and GSTT1 genes is very high in a number of populations and complete absence of these genes in individuals is common.

For a given DME assay that targets a gene that can be deleted or duplicated, the following genotyping results are possible:

- If both copies of a gene are deleted in a sample (copy number of 0), samples will not be amplified and will run with NTCs. If a sample running near the NTCs has been called as undetermined, that call can be manually adjusted to "noamp".
- If a sample contains 1, 2, or more than 2 copies of the gene and only one SNP allele is present, the samples will cluster together in a homozygous allele. Clusters will sometimes show some splitting: samples containing less target can run closer to the NTCs than those containing more copies of the target.
- If a sample carries more than 2 copies of a gene and both SNP alleles are present, it will fall within the heterozygous cluster or off to one side or the other of the cluster (that is, the heterozygous cluster may exhibit some spreading). If a sample close to the heterozygous cluster has been called as undetermined, manually adjust the call to heterozygous.

Analysis guidelines: SNP genotyping assays for triallelic SNPs

Triallelic SNPs are interrogated using two TaqMan® assays.

Example

- Assay 1: Major SNP allele (labeled with VIC™ dye) and first minor allele (labeled with FAM™ dye)
- Assay 2: Major SNP allele (labeled with VIC™ dye) and second minor allele (labeled with FAM™ dye).

Expected results

- Samples that are heterozygous for the detected SNP alleles will run as heterozygous with the assay designed to that mutant allele.
- Samples running in or near a homozygous cluster can be a true homozygote for the reported allele, or the sample can be a heterozygote for a reported allele and for the unreported SNP allele of a given assay. Samples with one reported allele in an assay can sometimes run together, or close to, those carrying two reported homozygous alleles. If a sample close to a homozygous cluster is called as undetermined, manually change the call to homozygous.
- Samples that are homozygous for the unreported SNP allele may cluster with NTCs or may exhibit weak amplification due to probe nonspecific activity. If a weakly amplifying sample is called as undetermined, manually adjust the call to 'noamp'.

(Optional) Export the TaqMan® Genotyper Software results, then import the files into AlleleTyper™ Software for translation using a biallelic translation specific for the triallelic SNP assay pair.

Manually determining genotype

If AlleleTyper™ Software is not available, use the table below to manually determine the sample genotypes. Note that the alleles reported by the ABCB1 SNP assays are given in the (+) strand genome orientation whereas the ABCB1 gene maps to the (-) genome strand. Thus, the reported SNP assay alleles and the SNP cDNA annotations are the reverse complement of one another.

Table 15 Translation table for the ABCB1 c.3095G>T/A triallelic SNP rs2032582 assays

Genotype	C/T assay c.3095G>A, A893S	C/A assay c.3095G>T, A893T
ABCB1 c.3095G>T/A	C_11711720D_40	C_11711720C_30
G/G	C/C	C/C
G/A	C/T	C/C
A/A	T/T	noamp
G/T	C/C	C/A
T/A	T/T	A/A
T/T	noamp	A/A

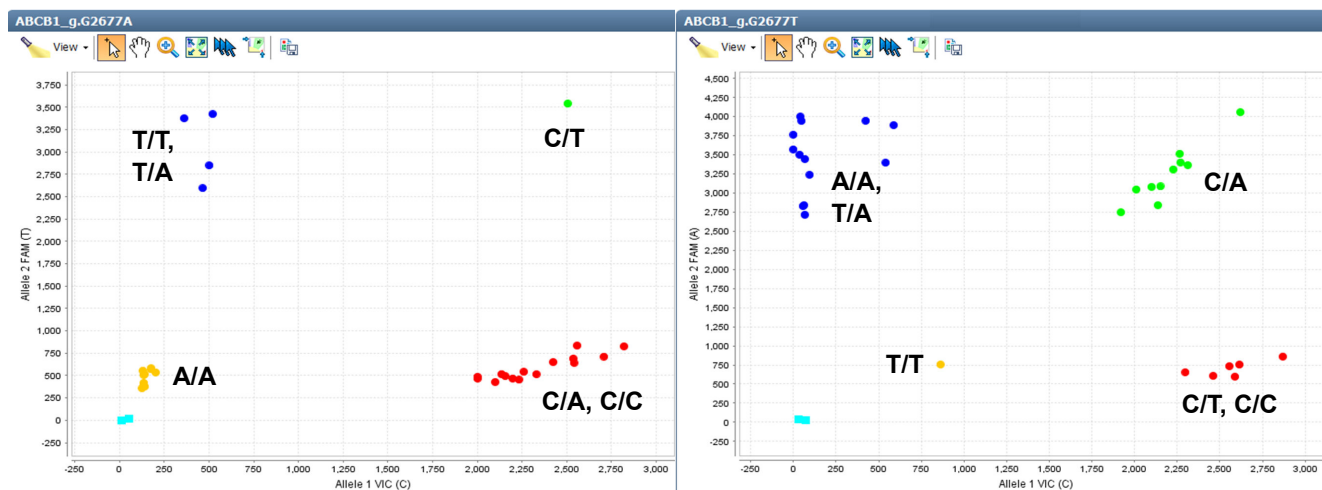


Figure 1 Triallelic SNP rs2032582 assay results

Left: ABCB1 c.3095G>A assay (C_11711720D_40) to the plus strand C wild type and T mutant allele. Right: ABCB1 c.3095G>T assay (C_11711720C_30) to the C wild type and A mutant allele.

Analysis guidelines: DME genotyping assays for adjacent SNPs

Pairs of assays are available for some adjacent SNP targets for which only three haplotypes are noted. For a given assay to one SNP allele:

- Samples that are heterozygous for the detected haplotypes will run as heterozygous with the assay designed to that haplotype.
- Samples running in or near a homozygous cluster can be a true homozygote for the reported haplotype, or the sample can be a heterozygote for a reported haplotype and an unreported haplotype of a given assay. Samples having just one reported haplotype may run together with, or close to, those carrying two reported homozygous haplotypes. If a sample close to a homozygous cluster is called as undetermined, manually change the call to homozygous.
- Samples that are homozygous for the unreported haplotype may cluster with NTCs or may exhibit weak amplification due to probe nonspecific activity. If a weakly amplifying sample is called as undetermined, manually adjust the call to "noamp".

Table 16 Translation table for the CYP2C19*2,*10 adjacent SNP assays

Haplotype/ Haplotype	Diploypes ^[1]	*2 assay 681 G/A - 680C	*10 assay 681G - 680 C/T
—	CYP2C19	C__25986767_70	C__30634128_10
G-C/G-C	*1/*1	G/G	C/C
G-C/G-T	*1/*10	G/G	C/T
G-C/A-C	*1/*2	G/A	C/C
G-T/G-T	*10/*10	noamp	T/T
G-T/A-C	*2/*10	A/A	T/T
A-C/A-C	*2/*2	A/A	noamp

^[1] Since the *2 and *10 alleles have not been observed to occur on the same chromosome, diploypes containing A-T haplotypes are not included.

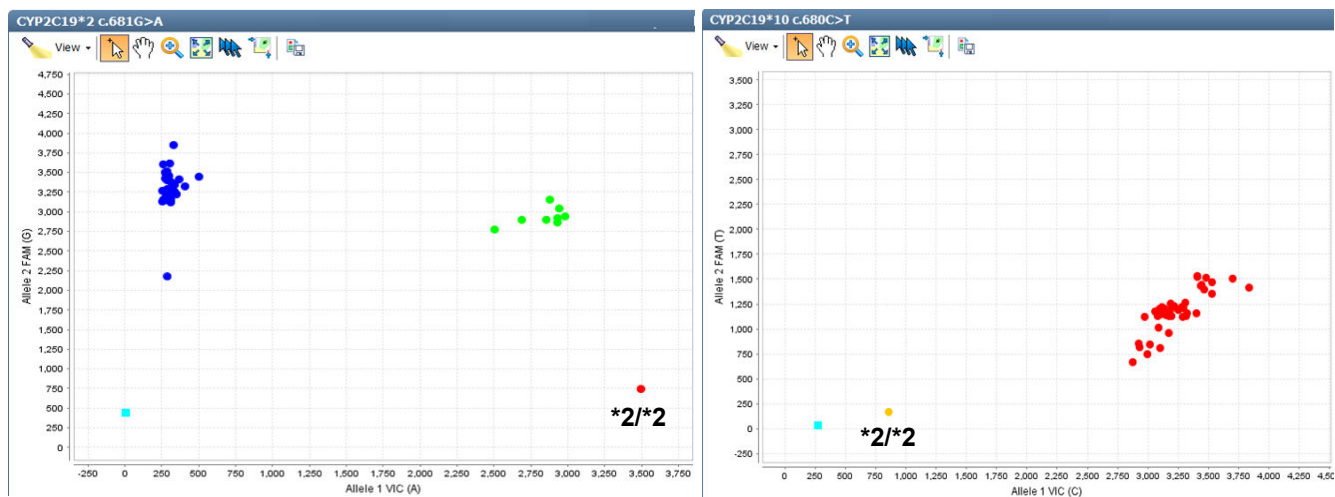


Figure 2 CYP2C19*2 and *10 assay OpenArray™ results

Left: rs4244285 CYP2C19*2 681G>A assay (C__25986767_70) to the G wild type and A mutant allele. Right: rs6413438 CYP2C19*10 c.680C>T assay (C__30634128_10) to the C wild type and T mutant allele. Samples: Coriell gDNAs; NA17263 has a *2/*2 diplotype that cannot be detected by the *10 assay.

Table 17 Translation table for the CYP2C9*3,*4 adjacent SNP assays

Haplotype/ Haplotype	Diploypes ^[1]	*3 assay 1075 A/C -1076T	*4 assay 1075A - 1076 T/C
—	CYP2C9	C__27104892_10	C__30634131_20
A-T/A-T	*1/*1	A/A	T/T
A-T/C-T	*1/*3	A/C	T/T
A-T/A-C	*1/*4	A/A	T/C
C-T/C-T	*3/*3	C/C	noamp
C-T/A-C	*3/*4	C/C	C/C
A-C/A-C	*4/*4	noamp	C/C

^[1] Since the *3 and *4 alleles have not been observed to occur on the same chromosome, diploypes containing A-A haplotypes are not included.

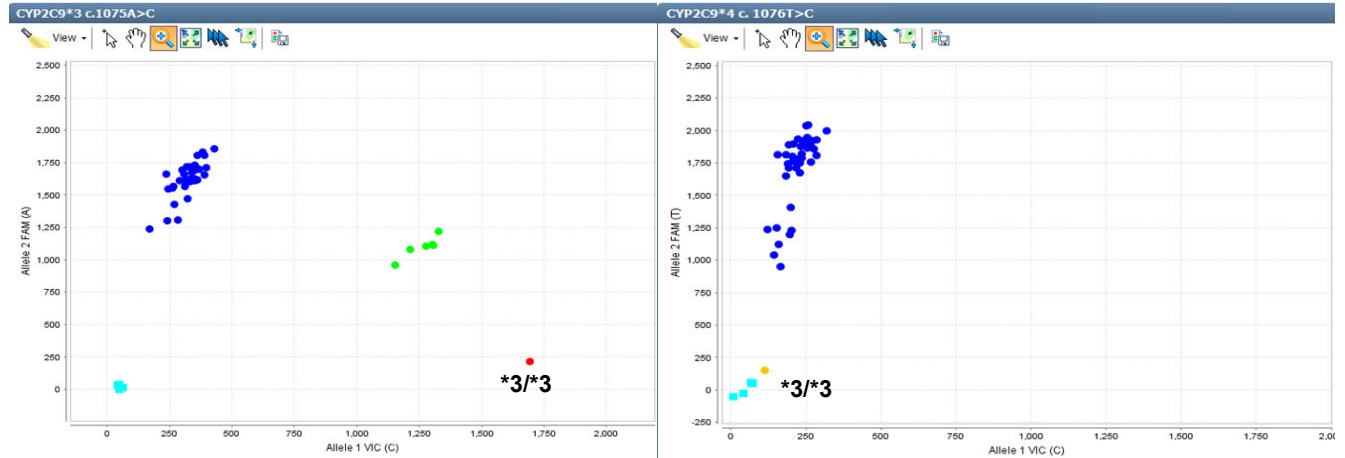


Figure 3 CYP2C9*3 and *4 assay OpenArray™ results

Left: rs1057910 CYP2C9*3 c.1075A>C assay (C__27104892_10) to the A wild type and C mutant allele. Right: rs56165452 CYP2C9*4 c.1076T>C assay (C__30634131_20) to the T wild type and C mutant allele. Samples: Coriell gDNAs; NA17247 has a *3/*3 diplotype that cannot be detected by the *4 assay. Note that the *3 heterozygous samples run in the lower portion of the split wild type FAM cluster in the *4 assay.

Related documentation

Title	Pub. No.
<i>TaqMan® Genotyper Software Getting Started Guide</i>	4448637
<i>TaqMan® OpenArray™ Genotyping Troubleshooting Guide</i>	MAN0011115

6

Prepare, run, and analyze PGx copy number experiments

- Introduction to copy number analysis 67
- How TaqMan® Copy Number Assays work 67
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- Set up PCR reactions 68
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- Analyze the run in the instrument software and export the results 70
- Analyze results in CopyCaller™ Software 71
- Review the copy number analysis data 72

This chapter contains brief procedures. For detailed procedures, refer to:

Title	Pub. No.
<i>TaqMan® Copy Number Assays Protocol</i>	4397425
<i>CYP2D6 Allele-specific Copy Number Analysis Quick Reference</i>	MAN0011114
<i>CopyCaller™ Software v2.0 User Guide</i>	4400042

Introduction to copy number analysis

Copy number variation must be assessed for DME genes that are known to exhibit copy number variation. For full details on running TaqMan® Copy Number Assay experiments, refer to the *TaqMan® Copy Number Assays Protocol* (Pub. No. 4397425). The protocol provides step-by-step instructions for performing and analyzing copy number variation quantitation experiments using TaqMan® Copy Number Assays and TaqMan® Copy Number Reference Assays for the ViiA™ 7 Real-Time PCR System. The same instructions apply for running experiments on the QuantStudio™ 12K Flex Real-Time PCR System.

How TaqMan® Copy Number Assays work

TaqMan® Copy Number Assays are run simultaneously with a TaqMan® Copy Number Reference Assay in a duplex real-time PCR reaction. The TaqMan® Copy Number Assay detects the target gene or genomic sequence of interest, and the TaqMan® Copy Number Reference Assay detects a sequence that is known to exist in two copies in a diploid genome (for example, the human RNase P H1 RNA gene).

The number of copies of the target sequence in each test sample is determined by relative quantification (RQ) using the comparative CT ($\Delta\Delta C_t$) method. This method measures the C_t difference (ΔC_t) between target and reference sequences, then compares the ΔC_t values of test samples to a calibrator sample(s) known to have two copies of the target sequence. The copy number of the target is calculated to be two times the relative quantity, because the human genome is diploid.

In a copy number quantification reaction, purified genomic DNA is combined with:

- The TaqMan® Copy Number Assay, containing two primers and a FAM™ dye-labeled MGB probe to detect the genomic DNA target sequence.
- The TaqMan® Copy Number Reference Assay, containing two primers and a VIC™ dye-labeled TAMRA™ probe to detect the genomic DNA reference sequence.
- One of the following master mixes:
 - (Recommended) TaqPath™ ProAmp™ Master Mix
 - TaqMan® Genotyping Master Mix

Reactions are run on an Applied Biosystems™ Real-Time PCR System. After amplification, data files containing the sample replicate C_t values for each reporter dye can be exported from the real-time PCR instrument software and imported into a software analysis tool. Applied Biosystems™ CopyCaller™ Software is recommended for post-PCR data analysis of copy number quantification experiments.

Procedural guidelines

- Use 10 ng of high-quality DNA per reaction.
- Run quadruplicate reactions for each DNA sample. The OA_Genotyping_CalcSheet assumes quadruplicate reactions in 384-well plates.
- For quality metrics calculations, each plate should contain enough samples such that there will be at least 7 samples with the same copy number.
- Include a no template control (NTC) reaction in each plate. In place of the DNA sample, use the same diluent used to dilute the DNA.
- Include one or more samples of known copy number as controls (reference or calibrator sample).

Before you begin

- Normalize the DNA samples: dilute each DNA sample to 5 ng/ μ L in nuclease-free water or 1X TE buffer in a total of 10 μ L.
Use the OA_Genotyping_CalcSheet to calculate the dilutions based on the concentration determined from the RNase P assay.
- Generate the CNV Sample .txt file from the OA_Genotyping_CalcSheet.

Set up PCR reactions

1. Prepare the reagents:
 - a. Completely thaw the assays, gently vortex, then briefly centrifuge to bring the contents to the bottom of the tube.
 - b. (*Large-scale copy number assays (60X) only*) Dilute the assay 1:3 (final concentration 20X) with 1X TE, pH 8.0.
For example, combine 10 μ L of 60X assay with 20 μ L of TE.
 - c. Swirl the master mix to mix contents thoroughly.

2. Prepare the reaction mix: combine the following components, invert or flick the tube to mix, then centrifuge briefly to bring the contents to the bottom of tube.
 - a. Combine the components according to the table.

Note: We recommend using four replicates of each sample.

Component	Volume per 10- μ L reaction ^[1]
TaqPath™ ProAmp™ Master Mix ^[2]	5.0 μ L
TaqMan® Copy Number Assay (20X)	0.5 μ L
TaqMan® Copy Number Reference Assay (20X)	0.5 μ L
Nuclease-free water	2.0 μ L
Total volume	8.0 μL

^[1] Use the OA_Genotyping_CalcSheet to calculate volumes for multiple reactions.

^[2] TaqMan® Genotyping Master Mix (2X) can also be used.

- b. Invert or flick the tube to mix, then centrifuge briefly.
3. Transfer 8 μ L of the reaction mix to the sample and control wells of a 384-well reaction plate.
4. Vortex the normalized gDNA samples (5 ng/ μ L), add 2 μ L of each sample or control to the appropriate wells of the plate, then mix by pipetting up and down several times.
5. Briefly centrifuge the plate to ensure the reaction mixes are at the bottom of each well and to minimize air bubbles.
6. Seal the plate with MicroAmp™ Optical Adhesive Film, ensuring that all 4 edges have a tight seal.

Run the reactions

1. Create an experiment in the instrument software with these properties:

Property	Setting
Experiment type	Standard Curve
Reagents	TaqMan® Reagents
Run speed	Standard

2. In the **Define** screen, enter the Target name, then select the appropriate Reporter and Quencher for the target.

Assay	Reporter	Quencher
TaqMan® Copy Number Assay	FAM™	MGB-NFQ
TaqMan® Copy Number Reference Assay	VIC™	TAMRA™

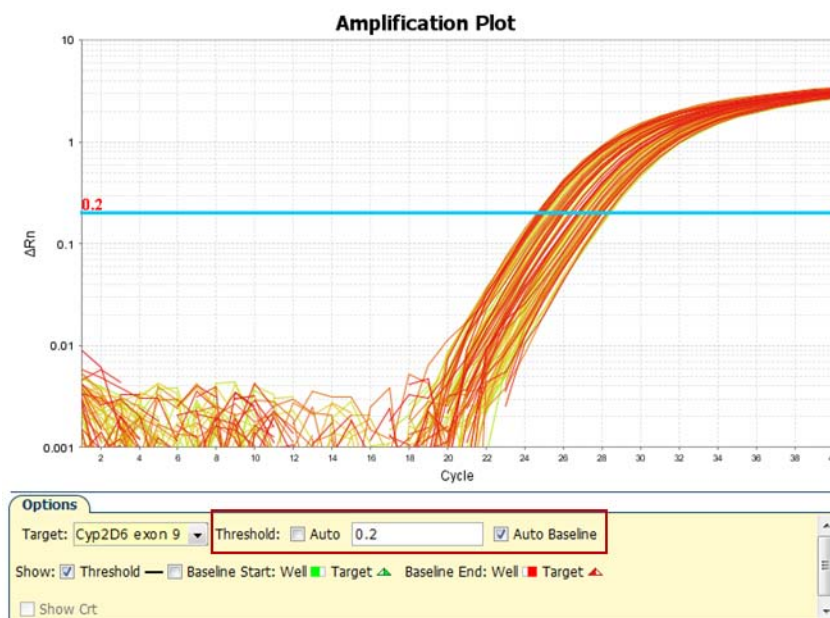
3. In the **Assign** screen:
 - Import the CNV Sample .txt file generated from the OA_Genotyping_CalcSheet, or manually assign sample names to the appropriate wells.
 - Assign both assays to each well.
4. In the **Run Method** screen:
 - Set the reaction volume to 10 μ L.
 - Confirm the following thermal cycling parameters.

Stage	Step	Temperature	Time
Hold	Step 1	95°C	10 min
PCR (40 cycles)	Step 1	95°C	15 sec
	Step 2	60°C	60 sec

5. Save the experiment, load the sealed plate into the instrument, then start the run.

Analyze the run in the instrument software and export the results

1. In the **Analysis** tab of the experiment, click **Analyze**.
2. In the **Amplification Plot** for each target, deselect **Auto Threshold**, then set the C_t threshold to **0.2**. Alternatively, use 0.1 as needed to ensure that the threshold is in the middle of the log phase.
 Keep **Auto Baseline** selected.




3. Click **Analyze**, then save the experiment.

4. In the **Export** screen, ensure that the only **Results** is selected.
5. Select a location and file name, select ***.txt** in the **File Type** dropdown list, then click **Start Export**.

Analyze results in CopyCaller™ Software

The recommended settings in CopyCaller™ Software are described. See “CopyCaller™ Software analysis options” on page 71 for other analysis options.

1. In the software, select **File ▶ Import**, select the file that was exported from the instrument software, then click **Open**.
2. In the **Assay Selection Table**, click row(s) to select one or more assays, then click  (Analysis Settings).

3. In the **Analysis Settings** window:
 - Select **With calibrator sample**
 - For **Calibrator Sample**: select **Median C_t**
 - For **Calibrator sample copy number**: enter **2**

Note: Enter 1 for GSTT1 and GSTM1 assays.

4. Click **Apply**.
The data are analyzed using the selected analysis settings.

CopyCaller™ Software analysis options

Analysis method	Description	How to select
Recommended: Median C _t	Copy number results are based on the copy number of the sample with the median C _t or median C _t value. This method works well when most samples have the same copy number.	<ol style="list-style-type: none"> 1. Select With Calibrator Sample. 2. In the Calibrator Sample Name drop-down list, select Median C_t
Most frequent copy number	Copy number results are based on the assumption that most samples have the same copy number.	<ol style="list-style-type: none"> 1. Select Without Calibrator Sample. 2. In the Most Frequent Sample Copy Number field, enter the expected value (Usually 2. Enter 1 for GSTM1 & GSTT1).

Analysis method	Description	How to select
Calibrator	<p>Copy number results are based on the copy number designated for one calibrator sample.</p> <p>Include a calibrator sample on each plate.</p> <p>IMPORTANT! The calibrator should be of the same sample type as the test samples.</p>	<ol style="list-style-type: none"> 1. Select With Calibrator Sample. 2. In the Calibrator Sample Name drop-down list, select or enter a sample to use as the calibrator. 3. [Optional] Change Calibrator Sample Copy Number (default = 2).

Review the copy number analysis data

1. In the **Assay Selection Table**, select the checkbox to the left of the analyzed assay to display the copy number analysis data.
2. Review the copy number analysis data to confirm that it meets the following criteria:

Criteria	Viewing tool
Samples have comparable VIC™ C _t values	Well and Results tables
Standard deviation is low for replicates (<0.15)	Results table
Calculated copy number values are close to integer values	Results table and Copy Number Plot
Confidence and Z-score values are acceptable for the predicted copy number calls	Results table
Copy number of control samples is as expected	Results table and Copy Number Plot
Copy number variation frequency is within the expected range	Results table and Copy Number Plot
Samples cluster into well-defined, well-separated copy number groups	ΔC _t plot

3. Review the quality metrics.

For sample copy number calls having confidence values ≥95%, consider passing or failing the call based on the |Z-score| value as shown in the table. |Z-score| is only evaluated for high confidence samples.

Z-score	Status
< 1.75	Pass
2.65 > z ≥ 1.75	Pass with caution
≥ 2.65	Fail

Passing the default 95% confidence threshold and |Z-score| of <1.75 is very achievable for good quality samples having 1–3 copies and is more difficult to achieve for lower quality samples carrying duplications or for higher copy samples.

Consider passing samples with calls of ≥ 3 copies that fall below the 95% confidence level if:

- The calculated copy numbers are close to integer values, and
- The samples cluster with passing samples of the same copy number group in the ΔC_t plot.



Perform translation analysis in AlleleTyper™ Software

- Introduction to AlleleTyper™ Software 74
- PGx translation tables 75
- Sources of PGx translation information and example translation tables 75

Introduction to AlleleTyper™ Software

AlleleTyper™ Software is an automated data analysis application that translates genetic pattern information from TaqMan® SNP Genotyping Assay and/or TaqMan® Copy Number Assay results to user-defined genotype or diplotype nomenclature. For complete instructions on how to use AlleleTyper™ Software and to create user-defined translation tables, see the *AlleleTyper™ Software User Guide* (Pub. No. 4486002).

AlleleTyper™ Software is a flexible tool used for conversion of sample genotype information for single or multiple genes or loci to the desired nomenclature, for example, the PGx gene-level star (*) allele nomenclature to describe SNP, InDel or copy number variant genotypes (for example, for the Cytochrome P450 genes). Star alleles are haplotypes that can contain multiple variants; these are associated with functional or nonfunctional gene products. AlleleTyper™ Software can translate the genotype results for special cases including triallelic or adjacent SNP interrogation using two TaqMan® SNP Assays, or the software can simply provide a name for a particular genetic outcome for a given SNP or copy number assay.

Before creating a translation table, or translator, consider all of the TaqMan® SNP Genotyping Assays (includes TaqMan® DME Genotyping Assays) and TaqMan® Copy Number Assays that will be used in your project, and which of these you want to include in a translation table for reporting sample genotype results using specific nomenclature. SNP assay and copy number assay experiment data generated on an Applied Biosystems™ real-time PCR system must be analyzed by TaqMan® Genotyper Software and CopyCaller™ Software, respectively, because results files exported from these software systems are input files for AlleleTyper™ Software.

AlleleTyper™ Software aids creation of translation tables by automatically converting monoallelic translation tables containing haplotype patterns to biallelic translators containing diplotype or genotype patterns. After examining biallelic translators for completeness and accuracy, they are imported into AlleleTyper™ Software along with TaqMan® Genotyper Software data files and/or CopyCaller™ Software data files. AlleleTyper™ Software then matches the sample genetic pattern data to patterns in the biallelic translator and reports back the designated nomenclature for each matching pattern, on a gene-by-gene basis when a multigene translator is used.

PGx translation tables

The translation tables provided by both Thermo Fisher Scientific and PharmGKB use the convention whereby the variant alleles are provided in the (+) strand orientation of the reference genome. Likewise, TaqMan® SNP and DME Genotyping Assays context sequences are provided in the (+) genome strand orientation. In contrast, the * allele nomenclature for many PGx genes does not follow this convention; the variant alleles might map to the (-) strand of the reference genome and thus are given in the reverse complement orientation (for example, CYP2D6, which maps to the (-) genome strand).

For successful translation of sample genotyping results by AlleleTyper™ Software, the base alleles used in the translation table must match the base alleles used in the TaqMan® Genotyper Software results files. In addition, the assay identifiers must match between the translation table and the TaqMan® Genotyper Software and/or CopyCaller™ Software results files.

Sources of PGx translation information and example translation tables

Thermo Fisher Scientific provides example AlleleTyper™ Software translation table template files and translation tables (including monoallelic and biallelic tables; single gene and multigene translators) for commonly tested drug metabolism gene variants. These files can be downloaded from the AlleleTyper™ Software web page at www.thermofisher.com/pgx and used as templates for creating translators specific to a TaqMan® Assays panel.

The PharmGKB website (www.pharmgkb.org) provides comprehensive haplotype translation tables for CYP gene variants found in the Human Cytochrome P450 (CYP) Allele Nomenclature Database (www.cypalleles.ki.se) as well as for other drug metabolism gene variants. These tables can be used as reference tables for creating AlleleTyper™ Software translation tables.



Troubleshooting




WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](https://www.thermofisher.com/support).

Troubleshoot with cycling and imaging run images

Many problems with OpenArray™ results can be diagnosed by examining the quality control (QC) images taken at various points during a cycling/imaging run.

The QC images are fluorescent or reflected light images taken before, during, and after cycling. They may require adjustment to make image features visible. To view the images, we recommend that you install the free software program ImageJ, which allows you to easily manipulate the images in ways that other image viewers cannot.

1. In the QuantStudio™ 12K Flex Software  **Export** screen:
 - a. Click **Browse** to select a uniquely-named folder for the QC images export.
 - b. Click **Export QC Images** (bottom of screen).

IMPORTANT! Select a new folder for images each time; exporting a second run to the same folder overwrites the images.

2. Use ImageJ to view the images of interest.

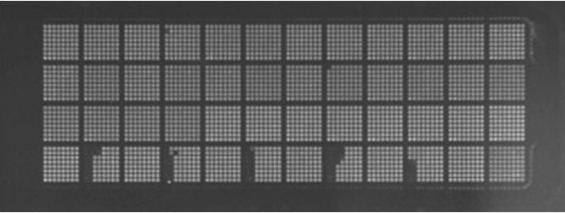
To...	View image...	Image description
Confirm the identity of images within a folder	BARCODE IMAGE.tiff	Reflected light image of the entire OpenArray™ plate
Evaluate the loading quality	PRE-READ_CHANNEL_4.tiff POST-READ_CHANNEL_4.tiff	Pre- and post-ROX™ images
Check for existing contamination on the case and/or heated cover	s00_c001_t01_p0001_m2_x3_e1_cp#_spotfind.tiff ^[1]	Pre-run reflected light spotfinding image (used by the software to determine the location of the holes)
Identify potential leaks or other contamination	s03_c001_t03_p0001_m2_x3_e1_cp#_spotfind.tiff ^[1]	Mid-run reflected light spotfinding image

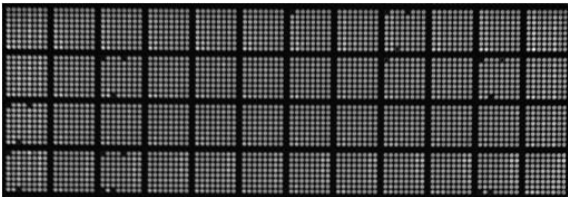
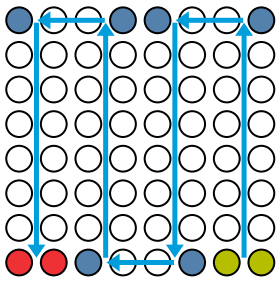
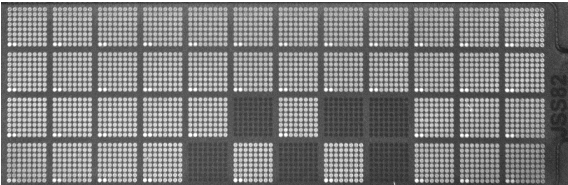
To...	View image...	Image description
Identify potential leaks or other contamination	s04_c001_t02_p0001_m2_x3_e1_cp#_spotfind.tiff ^[1]	Post-run reflected light spotfinding image
Look at patterns in the fluorescent data (for example, gradients)	STAGEx_CYCLEy_CHANNEL_z.tiff	FAM™ (z=1) or VIC™ (z=2) images at a particular cycle (y) of a particular stage (x) of the run.

^[1] The "cp#" in the image file name refers to the array position (1-4) within the QuantStudio™ 12K Flex instrument.

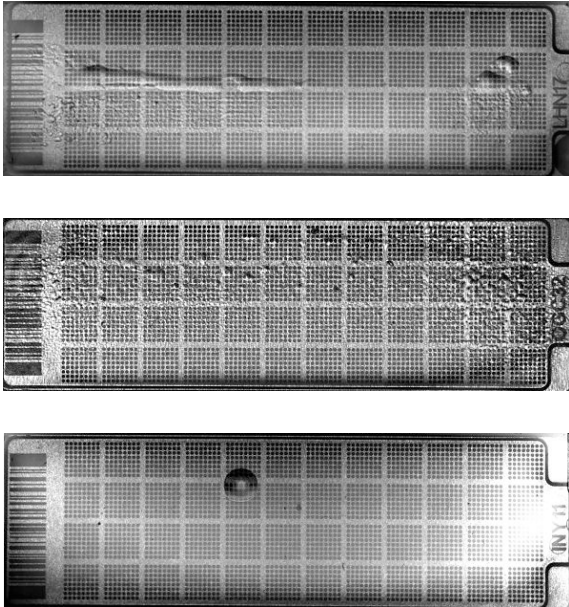
3. (Optional) Adjust the images for brightness and/or contrast to make image features visible.
 - a. Open the image in ImageJ.
 - b. Select **Image ▶ Adjust Brightness/Contrast** (or press **Ctrl+Shift+C**).
 - c. Click **Auto** or adjust the sliders until the features of interest in the image are visible.

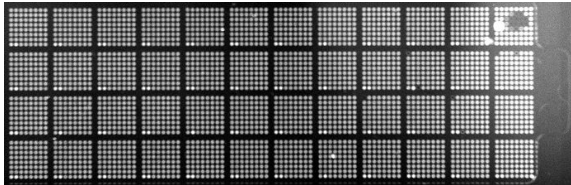
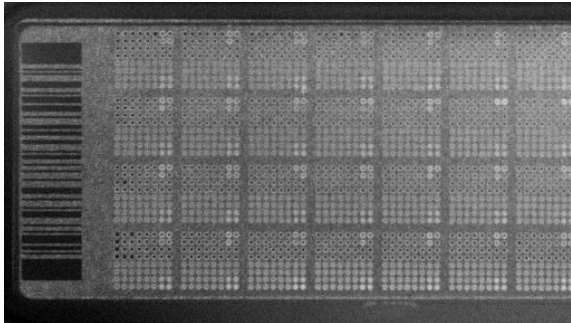
AccuFill™ instrument plate loading errors

Observation	Possible cause	Recommended action
There are empty through-holes 	Insufficient sample was added to the 384-well sample plate.	Use proper pipetting techniques. Ensure that there are no air bubbles in the pipette tips after sample aspiration.
	Reaction mix (sample + master mix) is not at the bottom of the 384-well sample plate.	Centrifuge the sample plate at 1,000 rpm for 60 seconds.

Observation	Possible cause	Recommended action
<p>Turn-holes are repeatedly missed</p> 	<p>AccuFill™ instrument is aligned too far to the left or to the right.</p> <p>Systematic loading problems can occur with the AccuFill™ instrument, which indicates a need for service. For example, when turn-holes are repeatedly missed across multiple subarrays, service is required. Turn-holes are where the AccuFill™ instrument changes direction during sample loading.</p>  <p>● Turn holes ● Start points ● Stop points</p>	<p>Contact your local field service engineer.</p>
<p>Entire subarrays are missing</p> 	<p>The sample/master mix not added to particular wells in the 384-well sample plate.</p> <p>Stuck tip mandrel on AccuFill™ instrument may need cleaning.</p> <p>Pipette tip not loaded on mandrel.</p>	<p>Visually inspect the sample plate to ensure that the wells have sample/master mix.</p> <p>Contact your local field service engineer.</p> <p>Contact your local field service engineer for frequent occurrences (infrequent occurrences can be due to a poorly molded tip).</p>

OpenArray™ plate assembly and handling errors

Observation	Possible cause	Recommended action
<p>Case leaks and bubbles inside the case</p>  <p>Improper sealing of the OpenArray™ plate in the OpenArray™ Case can lead to immersion fluid leaks or bubble formation inside the case, leading to uneven heating and imaging throughout PCR and to poor quality genotyping data.</p> <p>The images above are examples of OpenArray™ plates that have been affected by immersion fluid leaks. The images show where leaked fluid has condensed on the underside of the heated cover windows and obscured the view of the through-holes.</p> <p>The best image in which to detect leaks is the s03_c001_t03_p0001_m2_x3_e1_cp#_spotfind.tiff image. This image is taken at the start of cycling, which is where most leaks occur. See “Troubleshoot with cycling and imaging run images” on page 76.</p>	<p>Plate press was not engaged for at least 20 seconds.</p>	<p>Fully engage the plate press for at least 20 seconds.</p>
	<p>Damaged lid adhesive.</p>	<p>Remove the liner and visually inspect the lid adhesives for defects. Ensure that adhesive is not damaged or warped.</p>
	<p>Damaged fill port screw gasket.</p>	<p>Visually inspect the screw to ensure that the orange gasket is present and not damaged.</p>
	<p>Damaged fill port screw assembly. Breaks off too easily.</p>	<p>The screw may be mis-threaded: Unscrew it and use a new screw assembly.</p>
	<p>Oily lid or case from immersion fluid overflow.</p>	<p>Wipe off excess overflow of immersion fluid from the lid, case bottom, and crevices with 70% isopropyl alcohol, using a lint-free cloth (the cloth included with the OpenArray™ plate is acceptable).</p>
	<p>Immersion fluid exposed to air for too long.</p>	<p>Do not remove the immersion fluid syringe cap or draw air bubbles into the syringe until you are ready to load. Do not draw air bubbles into the syringe.</p>
	<p>Too large of a bubble inside the OpenArray™ case after sealing.</p>	<p>Minimize the size of the bubble by tilting the OpenArray™ case so that the fill port is at the highest point. Slowly fill the case with immersion fluid until only a small air bubble remains. Attach the screw and wipe off any excess oil that may have spilled onto the case.</p>
<p>Damaged plate press leading to uneven pressure.</p>	<p>Contact your field service engineer if you suspect that your plate press may be damaged.</p>	

Observation	Possible cause	Recommended action
<p>Sample blow out during the addition of immersion fluid</p> 	<p>The reactions in A12 were compromised during the addition of immersion fluid. Injecting the immersion fluid too quickly can actually purge the sample out of the through-holes near the fill port. Often this is caused by the user not purging the syringe slightly before use.</p>	<p>Dispense a small amount of immersion fluid onto a paper towel before use to ensure smooth operation of the syringe.</p>
<p>Evaporation of reaction mixture in through-holes</p> 	<p>Too much time elapsed before plate was sealed with lid and immersion fluid. In this example, the top half of each subarray was intentionally left open to the environment to demonstrate the effect of evaporation. "Donuts" are a result of the evaporated fluid in the through-holes.</p>	<p>Add immersion fluid as soon as the case is removed from the plate press to minimize the likelihood of evaporation, then seal the case with the lid.</p>

Recover from layout errors in the 384-well sample plate

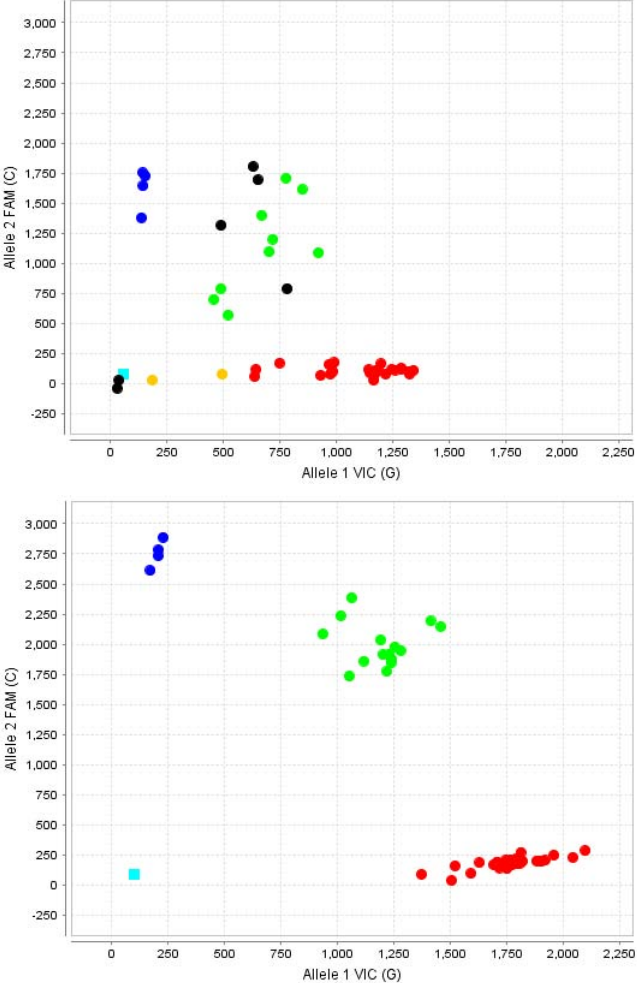
After the AccuFill™ procedure, you can recover from plate layout errors that were made during setup of the reactions in the 384-well sample plate. See the *OpenArray™ Sample Tracker Software Quick Reference* (Pub. No. 4460657) for additional information.

1. Create a corrected sample *.csv file.
2. Repeat "Generate 384-well sample plate layouts in the OpenArray™ Sample Tracker Software" on page 46, but select **OpenArray Plate X (for QuantStudio)** when exporting from the OpenArray™ Sample Tracker Software.
3. Import the corrected *.csv file into the QuantStudio™ 12K Flex Software.

Note: You can import either before starting the run or after the run is complete.

Troubleshooting unexpected genotyping results

The following table pertains to TaqMan® SNP Genotyping Assays run on OpenArray™ systems. For a comprehensive guide to troubleshooting SNP assay performance, refer to “Appendix A: Troubleshooting” in the *TaqMan® SNP Genotyping Assays User Guide*. (Pub. No. MAN0009593).

Observation	Possible cause	Recommended action
Diffuse clusters, loss of heterozygosity, and/or non-amplification for many samples in a dataset	Insufficient amount of starting DNA and/or PCR inhibitors present in the sample preparation.	<p>We recommend using 50 ng/μL starting DNA concentration. If it is not possible to increase the concentration of the starting material or remove inhibitors from the sample preparation, preamplify the sample. See the <i>TaqMan® OpenArray™ Genotyping Sample Preamplification User Bulletin</i> (Pub. No. MAN0011116).</p> <p>The images show poor quality SNP data (top) that is improved by preamplifying the samples (bottom).</p> 



Appendix A Troubleshooting

Troubleshooting unexpected genotyping results

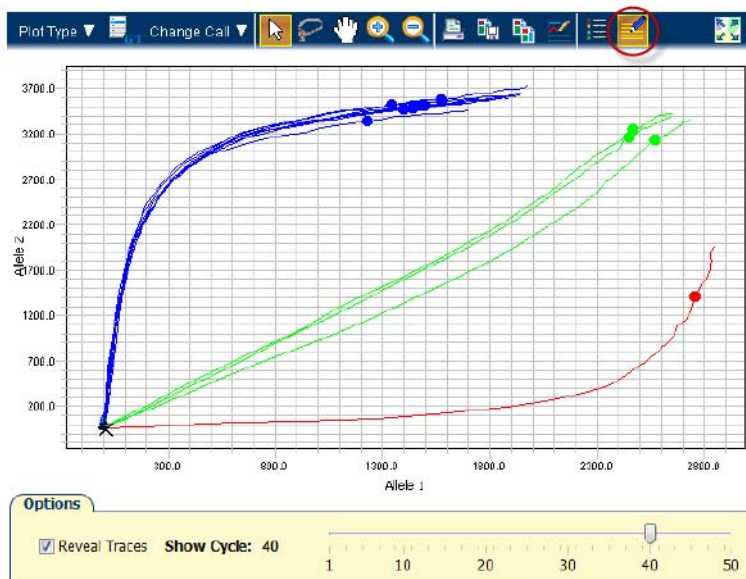
Observation	Possible cause	Recommended action
Amplification plot is missing expected cluster	Signal saturation caused software to call homozygous alleles as heterozygous (or <i>vice versa</i>).	Use real-time traces to manually call alleles. See "Recommended: Run OpenArray™ SNP genotyping experiments in real-time mode" on page 44 for more information.
Incorrect genotype called	Real-time traces were not used to identify outlier data points.	Use real-time traces to identify outlier data points and to manually call alleles. Real-time traces can be viewed in the QuantStudio™ 12K Flex Software or the Thermo Fisher Cloud Genotyping Module, which is accessed from thermofisher.com/cloud . See "Recommended: Run OpenArray™ SNP genotyping experiments in real-time mode" on page 44 for more information.

(Optional) Rerun samples that fail to cluster

If a sample fails to cluster well with other samples or fails to amplify properly with one or more assays (that is, there are UND and NOAMP calls in the run), rerun such samples using single tube DME assays on 96-well or 384-well plates. We recommend running control gDNA samples along with the unknown samples or use a reference panel (see “(Optional) Generate a reference panel” on page 58). The controls will help to provide more accurate genotype calling of the unknown samples.

Troubleshooting genotype calls for assays with merging clusters

It can be difficult for the secondary analysis software (for example, TaqMan® Genotyper Software) to make distinct genotype calls for assays with merging clusters (see example below). In these cases, use the real-time traces from the QuantStudio™ 12K Flex Software to review the data by assay and manually call data as necessary. Manually adjust the end-point cycle number to an earlier cycle using the Reveal Traces scroll bar; the clusters will have a greater degree of separation. Use this adjustment if any real-time traces do not show a smooth progression in signal throughout the run.



To manually adjust the the end-point cycle number:

1. Open the experiment.
2. Select **Analysis** ▶ **Allelic Discrimination Plot** from the **Experiment Menu** pane on the left.
3. Select the **Options** icon (circled in red).
4. Select **Reveal traces**.
5. Use the slider to adjust the end-point cycle number and optimize allele discrimination between genotype clusters.
6. Click **Analyze** to update genotype calls.



7. Save the run. Take note of the cycle number for each assay that has been adjusted. These numbers can later be entered into the TaqMan® Genotyper Software.

Note: A lower call cycle presently does not transfer to the TaqMan® Genotyper Software. The software will re-set the cycle number to 50 cycles. Set up a separate study with the lower cycle number.

8. (Optional) Select **Analysis ▶ QC Summary** from the **Experiment Menu** pane to view the QC flags for the run.

Click each flag to display its meaning, and a troubleshooting link appears in the right-side pane.

Note: Ignore the CTFAIL flag for genotyping data. Some samples do not have both alleles present. This flag displays the amplification curve from the absent allele does not meet quality metrics, but the amplification will be poor if an allele is absent.

Expected versus unexpected noamp and undetermined calls

For certain assays, it is expected that there will be some samples that legitimately fail to amplify or have an undetermined genotype call. These include samples run with assays that interrogate gene variants that are associated with copy number variation and with assays that detect triallelic SNPs or adjacent SNPs. See “Analysis guidelines: DME genotyping assays for genes in copy number variation regions” on page 61 and “Analysis guidelines: SNP genotyping assays for triallelic SNPs” on page 61 for details on how to analyze the data for such assays.

TaqMan® Drug Metabolism Genotyping Assays for genes in copy number variation regions

Some TaqMan® Drug Metabolism Genotyping Assays target polymorphisms in genes that exhibit copy number variation. Copy number variation analysis must be done in addition to genotyping with DME assays.

- If both copies of a gene are deleted in a sample (copy number of 0), samples will not be amplified and will run near or with the NTCs.
- If a sample contains more than 2 copies of a gene and both SNP alleles are present, it will fall within the heterozygous cluster or occasionally to one side or the other of it, in which case it may be called as undetermined. These should be manually called as heterozygous for data analysis purposes.



TaqMan® Drug Metabolism Genotyping Assays for triallelic SNPs and adjacent SNP targets

Triallelic SNP and adjacent SNP targets can be interrogated using a pair of Custom TaqMan® SNP Genotyping Assays. Each assay contains one probe for the major SNP allele or haplotype and one probe for one of the minor alleles or haplotypes. After running paired assays in separate reactions on the same genomic DNA samples, compare the results of the two assays to determine the sample genotype. For a given assay it is expected that:

- Samples containing one reported allele may run close to or within a homozygous cluster. Any samples running close to homozygous clusters that are called as undetermined should be manually called as homozygous for data analysis purposes.
- Samples that are homozygous for the unreported allele may cluster with NTCs or may exhibit weak amplification due to probe nonspecific activity. If a weakly amplifying sample is called as undetermined, manually adjust the call to "noamp".



Preamplification of low-concentration gDNA

Overview

Preamplification in OpenArray™ experiments helps to ensure sufficient template copies in the 33-nL qPCR reaction. At low template copy numbers, stochastic effects can dominate the reaction because the random events at each template molecule represent a large portion of the potential extension events. Stochastic events in qPCR reactions are generally dominant at levels of <10 template copies and negligible at levels of >100 template copies.

The standard OpenArray™ protocol for human SNP assays recommends starting with 50 ng/μL of genomic DNA. When diluted 50% in master mix and loaded into a 33-nL reaction chamber, the final template amount is 825 pg. This converts to 250 genomic copies (125 genomic copies for each allele in a heterozygote).

Preamplification can also be used to dilute PCR inhibitors from sample preparations that are of sufficient DNA concentration but contain impurities, and therefore do not amplify well with the TaqMan® OpenArray™ Genotyping Master Mix.

This protocol describes targeted multiplex preamplification of 1 to 256 SNP loci located in human genomic DNA samples. A multiplex pool of TaqMan® SNP Genotyping Assays is used to simultaneously preamplify up to 256 target polymorphisms in a single reaction using a reduced amount of input DNA sample.

The preamplification product can be used as the sample template input for SNP genotyping reactions with any of the individual TaqMan® SNP Genotyping Assays included in the multiplex preamplification assay pool. Perform genotyping experiments using the individual TaqMan® SNP Genotyping Assays following the standard protocol except substituting preamplified product for genomic DNA sample. It is not necessary to quantify or normalize preamplified product. The preamplified sample can be added directly into the reaction plate or further diluted in 1X TE Buffer to the desired concentration.

For specific applications, a custom OpenArray™ Preamp Pool specific for your TaqMan® SNP assay panel can be ordered along with your OpenArray™ plate (contact your sales representative for details).

Note: The protocol is compatible with TaqMan® SNP Genotyping Assays, but is not recommended for use with TaqMan® Copy Number Assays.

Guidelines for starting DNA sample concentration

DNA sample concentration	Preamplification	Recommendation
<0.4 ng/μL	Not recommended	Preamplification of DNA at concentrations <0.4 ng/μL is not recommended, due to the potential for stochastic events arising from low target number in the early rounds of the preamplification and qPCR reactions.
0.4 ng/μL to 4.0 ng/μL	Ideal preamplification range	The preamplification protocol was developed using genomic DNA samples within a starting concentration range of 0.4 ng/μL to 4.0 ng/μL and a total of 0.5 ng to 5 ng in the preamplification reaction. Optimal performance will be achieved if the starting concentration of genomic DNA samples is near the middle of the working range (2.5 ng/μL). Our recommended protocol for accurate quantification of human gDNA samples using the RNase P Detection Reagents Kit can be found in Appendix B of the <i>Pharmacogenomics Experiments User Guide</i> .
4 ng/μL to 25 ng/μL	User discretion	Samples at these concentrations may be preamplified without dilution, but these are out of the optimal range.
>25 ng/μL	Not recommended	Samples with DNA sample concentrations >25 ng/μL do not require preamplification. If samples at these concentrations do not amplify well with SNP assays, they likely contain PCR inhibitors. If this is the case, high concentration DNA samples may yield good results after dilution, and preamplification of these samples may be unnecessary.

Note: 1 ng of human genomic DNA = 300 genomic copies or 150 copies of each allele in a heterozygote.

Required materials

Unless otherwise indicated, all materials are available through **thermofisher.com**.
 MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Reagent	Cat. No.
TaqMan® PreAmp Master Mix	4391128
OpenArray™ PreAmp Pool	4485255 ^[1]
Genomic DNA Samples	Supplied by user
1X TE Buffer, pH 8.0	12090015
Nuclease-free water	MLS

^[1] Cannot be ordered online. For information on ordering an OpenArray™ Preamp Pool, contact your sales representative.

Perform the preamplification

1. Prepare the individual preamplification reactions as shown in the following table.

Note: It is convenient to use plates with well volumes that can accommodate a 20X larger volume than the reaction for the 20-fold dilution step after preamplification.

Note: TaqMan® PreAmp Master Mix and OpenArray™ PreAmp Pool can be prepared as a single reaction mix and distributed to the plate in 3.75-µL aliquots. These volumes can be increased 2-fold if more preamplified product is needed.

Component	Stock concentration	Final concentration	Volume
TaqMan® PreAmp Master Mix	2X	1X	2.5 µL
OpenArray™ PreAmp Pool	4X (0.20X each assay)	1X (0.05X each assay)	1.25 µL
Genomic DNA Sample	0.4 ng/µL to 4 ng/µL	0.1 ng/µL to 1 ng/µL (versus 150-1500 copies per µL)	1.25 µL
Total	—	—	5.0 µL

2. Firmly seal the reaction plate with a MicroAmp™ Clear Adhesive Film.
3. Vortex the reaction plate for 10 seconds and centrifuge briefly.
4. Run the preamplification cycling program on a GeneAmp™ PCR System 9700 (silver or gold block) or Veriti™ Thermal Cycler using the following thermal cycling conditions.

Stage	Step	Temp	Time
Hold	Activate	95°C	10 min
Cycling (10 to 14 cycles)	Denature	95°C	15 sec
	Anneal/Extend	60°C	4 min
Hold	Inactivate	99.9°C	10 min
Hold	—	4°C	up to 1 h or overnight

5. Transfer the reaction plate from the thermal cycler to a container with ice. Keep the plate on ice until you are ready to dilute the preamplified product.

Dilute and store the preamplified product

1. Centrifuge the reaction plate briefly prior to removing the film.
2. Remove the film, then add 95 μL of 1X TE Buffer to each well containing a preamplified product, to create a 1:20 dilution.
3. Seal the reaction plate with a new MicroAmp™ Clear Adhesive Film.
4. Vortex the reaction plate for 10 seconds and centrifuge briefly.
5. Store the preamplified product at -25°C to -15°C .



RNase P quantification for genotyping experiments

In this procedure, the quantification method uses TaqMan® Control Genomic DNA (human) as a calibrator instead of a standard curve. The OA_Genotyping_CalcSheet calculates the concentration of test DNA samples with this calibrator method.

Note: The OA_Genotyping_CalcSheet assumes duplicate RNase P reactions in a 384-well plate layout.

Download the OA_Genotyping_CalcSheet from thermofisher.com/oaqrc.

Required materials

Unless otherwise indicated, all materials are available through thermofisher.com.
MLS: Fisher Scientific (fisherscientific.com) or other major laboratory supplier.

Item	Source
TaqMan® Copy Number Reference Assay, human, RNase P (20X)	4403326
TaqMan® Control Genomic DNA (human; ready for use)	4312660
TaqPath™ ProAmp™ Master Mix ^[1]	4371357

^[1] Any TaqMan® Master Mix, including the TaqMan® Genotyping Master Mix can be used, but ensure that the run mode on the instrument is appropriate for the master mix.

Before you begin

- Dilute test DNA samples 1:10 in Nuclease-Free Water.
- Determine the total number of reactions required by including:
 - Diluted test DNA samples
 - Calibrator DNA sample
 - No template control (NTC) reactions; use Nuclease-Free Water in place of DNA sample

Note: We recommend a minimum of two reactions for each sample. The OA_Genotyping_CalcSheet is set up for duplicate reactions.

- Generate the RNase P Sample .txt file from the OA_Genotyping_CalcSheet.



Set up and run the PCR, then quantify the DNA

1. Prepare a reaction mix for the required number of reactions plus 10% overage.

Component	Volume per reaction ^[1]
TaqPath™ ProAmp™ Master Mix	5 µL
TaqMan® RNase P Assay (20X)	0.5 µL
Nuclease-free Water	2.5 µL
Total	8 µL

^[1] See the OA_Genotyping_CalcSheet to calculate volumes for multiple reactions.

2. Transfer 8 µL of the reaction mix to the wells of a 384-well PCR reaction plate.
3. Add 2 µL of sample DNA, control DNA, or Nuclease-Free Water to the appropriate wells.
4. Seal the plate with optical film.
5. Set up the real-time instrument with the following settings:
 - Experiment type: **Standard Curve**
 - Mode: **Standard**
 - Reporter: **VIC™**
 - Quencher: **TAMRA™**
 - Passive reference: **included**
6. To assign the sample names, import the RNase P Sample .txt file generated from the OA_Genotyping_CalcSheet.
7. Load the plate, then start the run.
8. After the run is complete, deselect all but the **Results** tab, select a file location, then export the results.
9. Remove duplicate sample IDs from the exported data by opening the exported data file in Excel™, then:
 - a. Copy-paste sample names and their corresponding C_t mean values into a new sheet.
 - b. Select both columns and use the **Remove Duplicates** function.
10. Calculate the concentration of test DNA samples:
 - a. Copy- paste the Sample Names and Mean C_t values into the OA_Genotyping_CalcSheet.
 - b. Enter the dilution factor of the test DNA samples into the OA_Genotyping_CalcSheet.



Expected performance and system specifications

See the QuantStudio™ 12K Flex System Product Bulletin (Pub. No. CO23802) for more detail on performance and specifications.

Table 18 Specifications for the QuantStudio™ 12K Flex OpenArray™ AccuFill™ System

Description	Specification
Fill rate	99.25%
Maximum allowable sample carryover	<1%
Maximum allowable assay carryover	<1%
Maximum number of through hole data points lost due to evaporation	<5%

Table 19 Specifications for QuantStudio™ 12K Flex TaqMan® OpenArray™ Genotyping Plates

Description	Specification
Assay conversion rate from a 7900HT Real-Time PCR system	>95% ^[1]
Concordance with assays run on a 7900HT Real-Time PCR system	99.7% ^[1]
Call rate	95% ^[1]
Number of haploid copies of gDNA/through-hole	250
TaqMan® OpenArray™ Genotyping Plate capacity per 8 hour day by a single person	12
Time from purified DNA to genotyping data	~5 hours
Throughput of one technician in one day	110,000

^[1] Individual performance is dependent on the integrity and purity of samples.



Controls for genotyping and copy number experiments

Overview

Laboratories can verify the performance of selected TaqMan® SNP Genotyping Assays and TaqMan® Drug Metabolism Genotyping Assays on OpenArray™ plates by using samples that represent all three genotypes (homozygous major allele, homozygous minor allele, and heterozygous) to test for both amplification and cluster separation. A laboratory may prefer to use samples that represent at least two genotypes (homozygous major and minor alleles, or homozygous major allele and heterozygous) to test that both assay probes function. Ideally, gDNA samples of known genotypes are used for such experiments. However, for rare DME variants that are not well-represented in populations, it may be necessary to use synthetic templates.

This Appendix includes information on sources of control cell line sample DNAs for DME and SNP genotyping assays, as well as for copy number assays. It also includes information on ordering synthetic template DNAs in plasmids.

Sources of reference materials

This section describes some publicly available sources for reference or control materials that can be used in genetic testing and assay performance experiments.

TaqMan® Drug Metabolism Genotyping Assays test data

All TaqMan® Drug Metabolism Genotyping Assays were run on 180 unique DNA samples from four different populations in assay performance and reproducibility testing. Samples tested included a panel of 45 African American and 45 Caucasian samples obtained from the Coriell Cell Repositories and a panel of 45 Japanese and 45 Chinese samples provided by a collaborator. The minor allele frequencies determined for each assay are provided at thermofisher.com (assay search results) and in the DME Index.

Note: Many of the polymorphisms interrogated by this assay collection have very low minor allele frequencies (MAFs) of less than 1%; thus, the minor allele was not detected for all assays within the 180 test samples.

For TaqMan® Drug Metabolism Genotyping Assays having a reported Applied Biosystems™ MAF in the African American or Caucasian populations, heterozygous or minor allele homozygous samples may be used as reference or control samples in genetic testing and assay performance studies. A complete list of the samples used and their genotypes is available at thermofisher.com.

Order the gDNA samples from the Coriell Cell Repositories at <http://ccr.coriell.org>. Please note that the genotypes of these samples were, for the most part, not verified by sequence analysis.

NCBI dbSNP genotype data

Another source of DME assay control samples is the refSNP submissions in the NCBI database of Short Genetic Variations (dbSNP) at www.ncbi.nlm.nih.gov/SNP.

Search the database by rs ID to navigate to specific refSNP pages that may have genotype information for variants of interest, noted in the Allele summary box or the Population Diversity table. Cell line gDNA samples are available from the Coriell Institute biorepositories (<https://catalog.coriell.org>) for samples sequenced in the 1000 Genomes project or genotyped in the International HapMap project.

- 1000g controls: if the Allele summary box contains MAF/MinorAlleleCount information from the 1000 Genomes project, navigate to the 1000 Genomes browser (www.ncbi.nlm.nih.gov/variation/tools/1000genomes) and search by rs ID. This will return a genotype results table for the SNP and neighboring SNPs for all 2500 samples used in the project, grouped by population. Expand a population to view individual samples and their genotypes
- HapMap controls: if the Population Diversity table contains genotype information from the HapMap project, the sample genotypes can be found by clicking on the submission ss # to navigate to the submission details page. For HapMap samples, the sample genotypes can also be found at the HapMap web site (<http://hapmap.ncbi.nlm.nih.gov>). A subset of the HapMap samples were sequenced by the 1000g project.

Centers for Disease Control and Prevention (CDC) reference materials

Clinical Laboratory Improvement Amendments (CLIA)

wwwwn.cdc.gov/clia/Resources/GETRM/default.aspx

The Centers for Disease Control and Prevention (CDC) provides genetic information on cell line DNAs that can be used as reference materials for genetic testing and assay validation. Some of these cell lines were characterized by the Genetic Testing Reference Materials Coordination Program (GeT-RM). One major focus category is the 'Genetic Inherited Disease & Pharmacogenetics' section. Download tables of reference samples that contain PGx/DME or disease allele variants, many of which have been confirmed by multiple labs and genetic testing technologies, can be downloaded from: wwwwn.cdc.gov/clia/Resources/GETRM/MaterialsAvailability.aspx

TaqMan® Copy Number Assays for DME genes

Several DME genes occur in CNV regions (see "DME genes and copy number variation" on page 15). In addition, the CYP2D6 and CYP2A6 genes are known to recombine with related pseudogene sequences to generate hybrid genes, many of which have decreased or null gene activity. TaqMan® Copy Number Assays can be used to detect deletions, duplications, and hybrid gene alleles. TaqMan® Copy Number Assays for the major known DME genes that exhibit CNV have been pre-tested on a panel of 45 each African American and Caucasian (the same samples as were used for TaqMan® Drug Metabolism Genotyping Assay validation studies). A complete list of the samples used and their genotypes is available at www.thermofisher.com/pgx.

The gDNA samples can be ordered from the Coriell Cell Repositories website (<http://ccr.coriell.org>).

Plasmid controls

We have successfully used synthetic major and minor allele template DNAs cloned into plasmids to test the performance of over 300 TaqMan® DME Assays. In addition to major or minor allele sequences, plasmids carry the RNase P RPPH1 gene for accurate quantitation of plasmids by the standard curve analysis before use in genotyping experiments. Equal quantities of major and minor allele plasmids are mixed together to create heterozygous controls. Use the homozygous and heterozygous plasmid controls to demonstrate amplification and detection of all three genotypes by a given assay. Plasmid control samples will often, but not always, cluster with gDNA samples. Review example data in the TaqMan® Drug Metabolism Genotyping Assays on OpenArray™ Plates .ppt file from: www.thermofisher.com/pgx.

For information on ordering TaqMan® DME and SNP Genotyping Assay plasmid controls, email QuantStudioFrontDesk@lifetech.com.



Good PCR practice

Prevent contamination and non-specific amplification

PCR assays require special laboratory practices to avoid false positive amplifications. The high throughput and repetition of these assays can lead to amplification of one DNA molecule.

PCR good laboratory practices

When preparing samples for PCR amplification:

- Use a positive-displacement pipette or aerosol-resistant pipette tips.
- Follow proper pipette-dispensing techniques to prevent aerosols.
- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
 - Sample preparation
 - PCR setup
 - PCR amplification
 - Analysis of PCR products
- Do not bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Centrifuge tubes before opening. Avoid splashing or spraying PCR samples.
- Keep reactions and components capped as much as possible.
- Clean lab benches and equipment periodically with 10% bleach solution. Use DNAZap™ PCR DNA Degradation Solutions Cat. No. AM9890).



Safety



WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
 - Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the “Documentation and Support” section in this document.
-

Chemical safety



WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the “Documentation and Support” section in this document.
 - Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
 - Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
 - Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
 - Handle chemical wastes in a fume hood.
 - Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
 - After emptying a waste container, seal it with the cap provided.
 - Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
 - Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
 - **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.
-

Biological hazard safety



WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
www.cdc.gov/biosafety/publications/bmb15/BMBL.pdf
 - World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf
-

Documentation and support

Related documentation

Document	Pub. No.
<i>Thermo Scientific™ KingFisher™ Flex User Manual</i>	N07669
<i>MagMAX™ DNA Multi-Sample Ultra Kit MagMAX™ Express-96 Magnetic Particle Processor Protocol</i>	4428202
<i>Best Practices for Collection of Buccal Swabs for Genotyping Experiments</i>	MAN0014348
<i>Isolation of DNA for Genotyping Experiments</i>	MAN0014561
<i>OpenArray™ Sample Tracker Software Quick Reference</i>	4460657
<i>OpenArray™ SNP Genotyping Experiments</i>	MAN0014351
<i>OpenArray™ Genotyping Analysis</i>	MAN0014352
<i>TaqMan® OpenArray™ Genotyping Troubleshooting Guide</i>	MAN0011115
<i>TaqMan® Genotyper Software Getting Started Guide</i>	4448637
<i>TaqMan® SNP Genotyping Assays User Guide</i>	MAN0009593
<i>TaqMan® Drug Metabolism Assays for Triallelic SNPs Application Note</i>	135AP01-01
<i>AlleleTyper™ Software User Guide</i>	4486002
<i>Copy Number Analysis for Pharmacogenomics Experiments</i>	MAN0014350
<i>RNase P Quantification for Genotyping Experiments</i>	MAN0014349
<i>Creating Standard Curves with Genomic DNA or Plasmid DNA Templates for Use in Quantitative PCR Application Note</i>	4371090
<i>TaqMan® Copy Number Assays Protocol</i>	4397425
<i>CYP2D6 Allele-specific Copy Number Analysis Quick Reference</i>	MAN0011114
<i>Copy Number Analysis for Pharmacogenomics Experiments</i>	MAN0014350
<i>CopyCaller™ Software v2.0 User Guide</i>	4400042

Document	Pub. No.
<i>Applied Biosystems™ QuantStudio™ 12K Flex Real-Time PCR System OpenArray™ Experiments User Guide</i>	4470935
<i>OpenArray™ technology on the QuantStudio™ 12K Flex System Product Bulletin</i>	C023802
<i>QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide</i>	4470689
<i>QuantStudio™ 12K Flex Real-Time PCR System: OpenArray™ Plate Quick Reference</i>	4478673
<i>QuantStudio™ 12K Flex Software Help</i>	—

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- Product support, including:
 - Product FAQs
 - Software, patches, and updates
 - Training for many applications and instruments
- Order and web support
- Product documentation, including:
 - User guides, manuals, and protocols
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.

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