applied biosystems

Pharmacogenomics Experiments

APPLICATION GUIDE

TaqMan™ Genotyping and Copy Number Assays

for use with:

QuantStudio™ 12K Flex Real–Time PCR System with OpenArray™ block (QuantStudio™ 12K Flex OpenArray™ AccuFill™ System)

TaqMan™ OpenArray™ Genotyping Plates

TaqMan™ Genotyper Software

CopyCaller™ Software

Catalog Numbers 4471090, 4488847

Publication Number MAN0009612

Revision E

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Revision history: MAN0009612 E (English)

Revision	Date	Description
E	24 September 2024	• The following updates were made throughout the document: – Vortex instructions were updated for setting up PCR reactions. – Sealing instructions were updated for sealing the OpenArray™ Plate. – Minor verbiage updates. • The support email was updated for ordering TaqMan™ DME and SNP Genotyping Assay plasmid controls ("Plasmid controls" on page 133). • The instrument image on the cover page was updated.
D.0	23 May 2022	• The TaqMan™ OpenArray™ PGx Panel, QuantStudio™ 12K Flex Real–Time PCR System (Cat. No. 4475395) was removed from the fixed TaqMan™ OpenArray™ PGx panel plates product list. • For the DNA isolation procedure, the MagMAX™ DNA Multi-Sample Ultra Kit was replaced with the MagMAX™ DNA Multi-Sample Ultra 2.0 Kit (Cat. No. A36570). • The following updates were made to the list of required materials for the OpenArray™ Plate workflow: – Clear plates were added as an option for the OpenArray™ 384-well Sample Plates. – The Biomek™ Seal and Sample Foil Lids were changed to an optional material. – A sharp edge, a blade, or a scalpel was added. – Nuclease-free water was added. – The ethanol was updated to 100% molecular grade ethanol. • The location of the sample layout file was corrected for OpenArray™ Sample Tracker Software (applies to OpenArray™ AccuFill™ Software v1.2 only). • The Biomek™ Seal and Sample Foil Lids were changed to an optional material. • A note was added to recommend a pipetting overage when setting up the PCR reactions. • The centrifuge speed for the OpenArray™ Plate workflow and troubleshooting empty through-holes was updated. • A new chapter was added for preparing genotyping experiments using EDT files (see Chapter 5, "Prepare and run OpenArray™ PGx SNP genotyping experiments using EDT files"). • Separate sections for preparing OpenArray™ Plates with OpenArray™ AccuFill™ Software v1.2 and OpenArray™ AccuFill™ Software v2.0 were added (see Chapter 4, "Prepare and run OpenArray™ PGx SNP genotyping experiments" and Chapter 7, "Prepare, run, and analyze PGx copy number experiments"). • The chapter for performing translation analysis in AlleleTyper™ Software was removed.
C.0	28 September 2016	• Ordering information incorporated into Chapter 2. • DNA isolation procedures moved into Chapter 3. • gDNA preamplification moved to new Appendix B. • Prepare, run, and analyze OpenArray™ PGx experiments split into two chapters. • New troubleshooting sections.
B.0	4 August 2014	• 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.

Revision	Date	Description
A.0	21 January 2014	New document.

The information in this guide is subject to change without notice.

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1 About pharmacogenomics experiments

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

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

OpenArray™ Plate PGx SNP genotyping experiments

Isolate DNA using the MagMAX™ DNA Multi-Sample Ultra 2.0 Kit (page 32)

(Optional) **Preamplification of low-concentration gDNA** (page 124)

Prepare and run OpenArray™ PGx SNP genotyping experiments (page 44)

OR

Prepare and run OpenArray™ PGx SNP genotyping experiments using EDT files (page 72)

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OpenArray™ Plate PGx copy number experiments

Isolate DNA using the MagMAX™ DNA Multi-Sample Ultra 2.0 Kit (page 32)

Prepare, run, and analyze PGx copy number experiments (page 108)

2 Background and tools for assay selection

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 28.

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 1 DME allele nomenclature websites

Gene family	Allele nomenclature website
CYP—Cytochrome P450 (CYP) genes	**https://www.pharmvar.org/**
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 28.

TaqMan™ Drug Metabolism Genotyping Assays

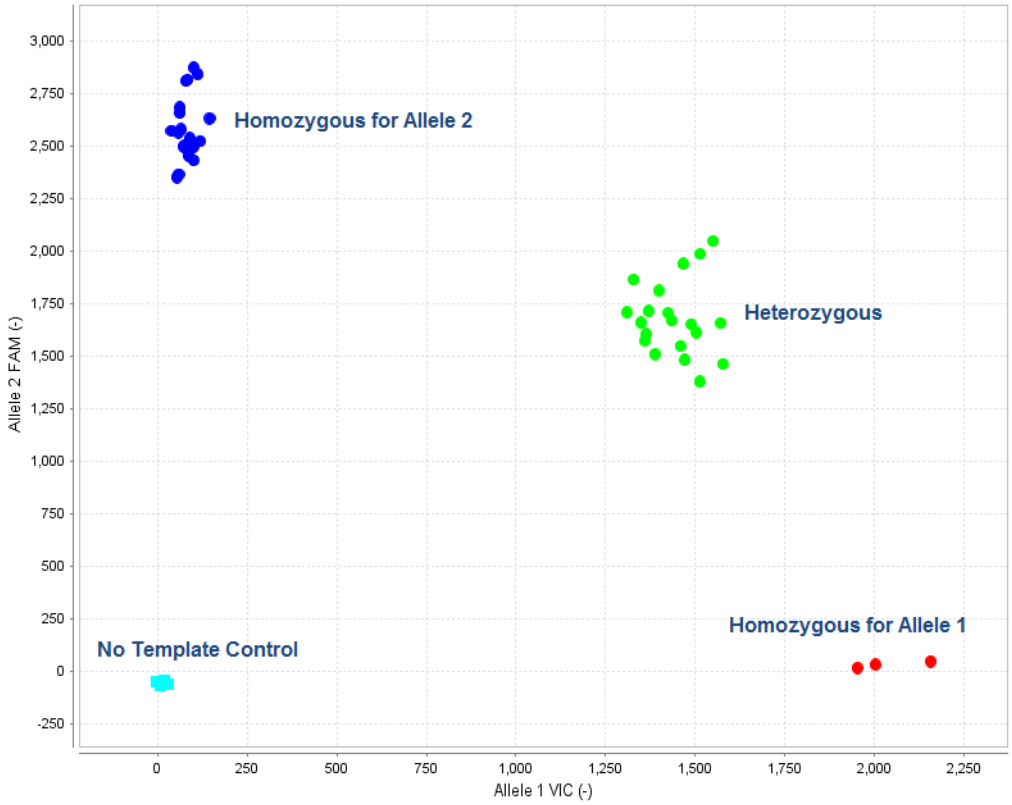
The TaqMan™ Drug Metabolism Genotyping Assay, a collection of approximately 2,700 assays, detects potentially causative SNP, MNP or InDel polymorphisms in 221 drug metabolism enzyme (DME) and associated transporter genes. The DME assays were designed using an optimized DME assay design algorithm that uses a high level of bioinformatics to enable 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 facilitate 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 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 12, on page page 103 for analysis.

Table 2 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 15).

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 3 DME Assays for genotyping adjacent SNPs

Gene	SNP ID	TaqMan™ DME assay	Allele name
CYP2C19	rs4244285	C__25986767_70	CYP2C19*2 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 (**https://www.pharmvar.org/gene/CYP2C19**): 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 103.

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 18). 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 30) 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 4. These assays were run on 90 Coriell gDNA samples from African American and Caucasian populations (the same panel used for DME Assay validation).

Table 4 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 (**https://www.pharmvar.org/**). 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 18):

Table 5 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 6).

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 6), 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 6 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 (**https://www.pharmvar.org/gene/CYP2D6**) 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 (**https://www.pharmvar.org/**) 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 18 for the complete list).

Table 7 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 heterogyous 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™ Assay 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 (MNPs) 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 22), 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

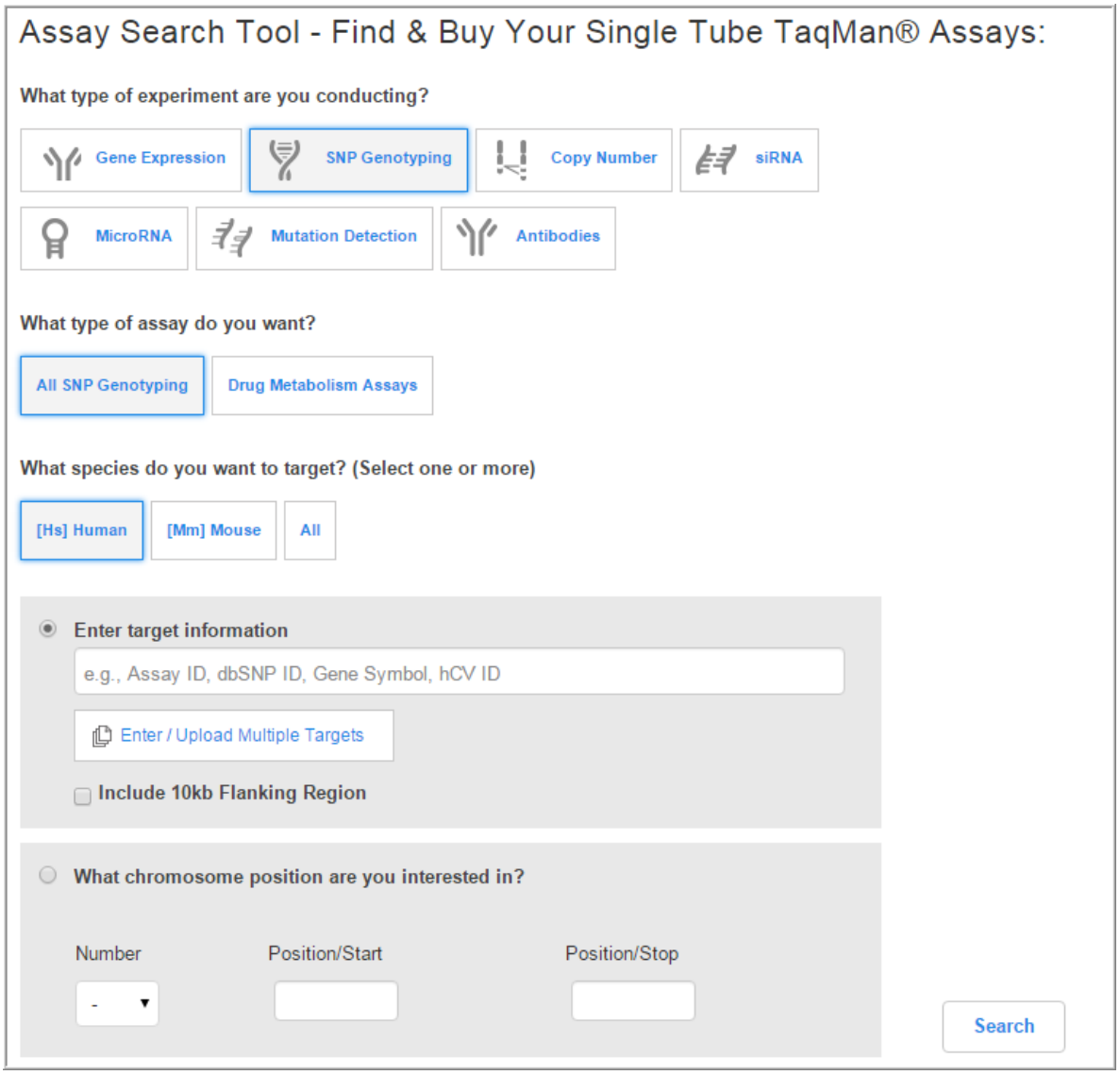
- Assay search tool available at **www.thermofisher.com/ordertaqman**
- TaqMan™ Drug Metabolism Genotyping Assays Index available at **www.lifetechnologies.com/taqmanandme**
- PharmaADME Core Marker Set available at **http://tools.lifetechnologies.com/content/sfs/brochures/cms_082106.xls**
- PGx Common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



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:

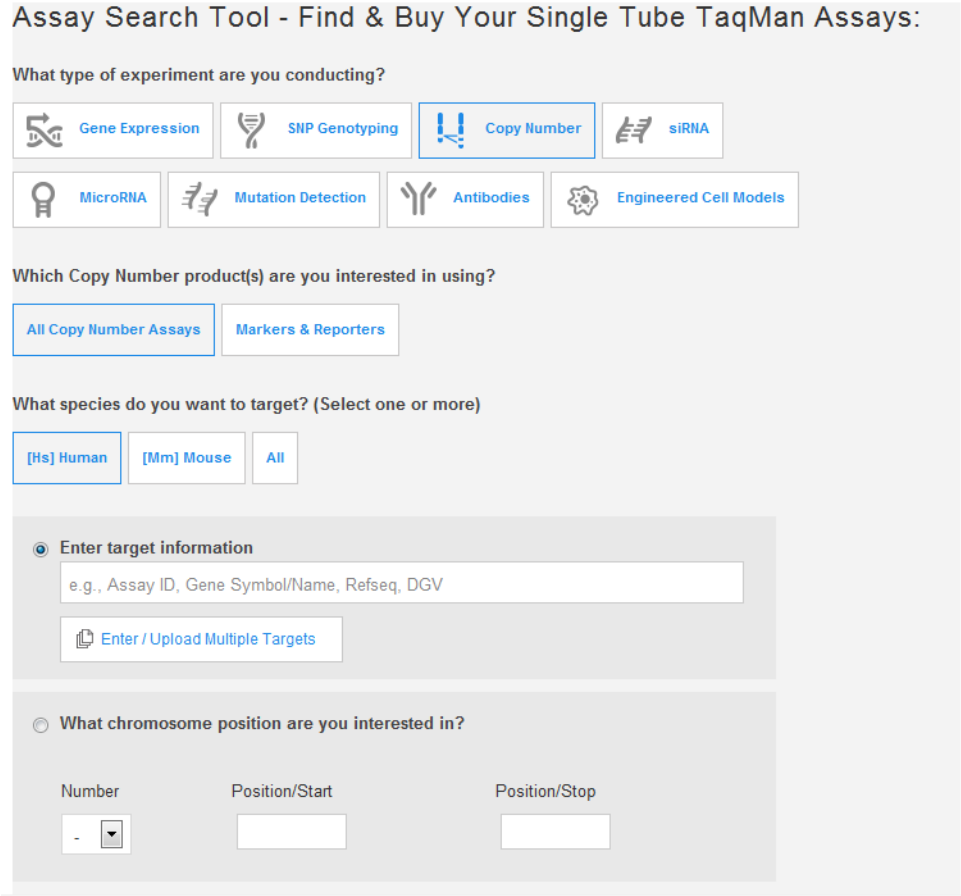
   - Read any **Important Information** notes associated with the assay (select **Important Information** next to the Assay ID). Notes provide information required to make ordering decisions and analyze data.
   - The annotation for triallelic SNP assays is associated with the SNP ID and not to the assay. 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**.



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.

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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 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

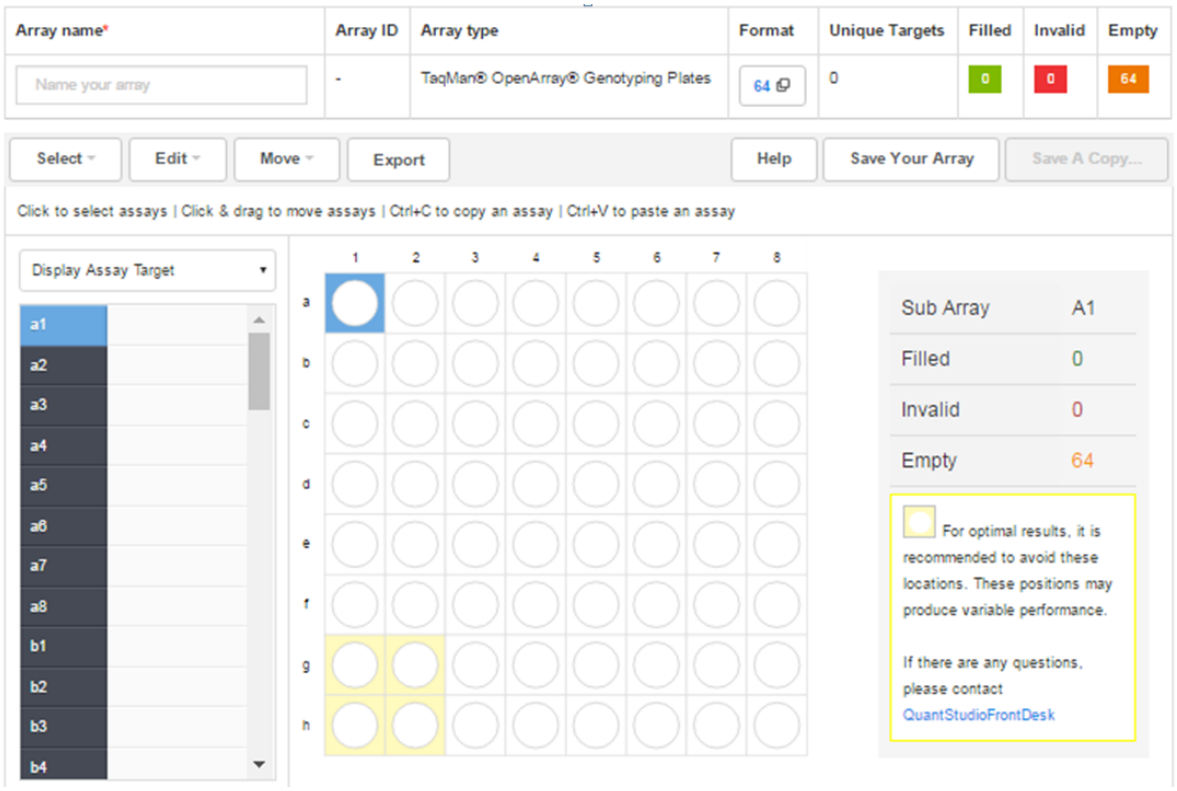
Fixed TaqMan™ OpenArray™ PGx panel plates

TaqMan™ OpenArray™ PGx panel available for use:

- TaqMan™ OpenArray™ PGx Express Panel, QuantStudio™ 12K Flex Real–Time PCR System (Cat. No. 4488847)

Order custom OpenArray™ plates

1. Go to **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.



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 8 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 9 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 10 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 2.0 Kit

Kit contents and storage

Reagents provided in the kit are sufficient for 100 reactions.

Table 11 MagMAX™ DNA Multi-Sample Ultra 2.0 Kit (Cat. No. A36570)

Component	Quantity	Storage
Enhancer Solution	4.5 mL	15–30°C
Proteinase K	4.5 mL	
Binding Solution	45 mL	
DNA Binding Beads	4.5 mL	
Wash I Solution	110 mL	
Elution Solution	12 mL	

For 1,000 reaction volume use Cat. No. A36578 (Proteinase K), A36579 (DNA Binding Beads), A36580 (Wash I Solution), A36581 (Lysis/Binding Solution), A36582 (Elution Solution), and A36583 (Enhancer Solution).

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Instrument	
Magnetic particle processor	
KingFisher™ Flex Magnetic Particle Processor 96DW with 96 deep-well head	5400630
Consumables	
Deep-well plates, one of the following:	
KingFisher™ 96 Deep-Well Plates, v-bottom, polypropylene	95040450
KingFisher™ 96 Deep-Well Plates, barcoded	95040450B
96-well standard plate (elution step)	
KingFisher™ 96 KF microplate	97002540
Tip comb, compatible with the magnetic particle processor	
KingFisher™ 96 tip comb for DW magnets	97002534
Equipment	
Incubator with metal racks	**MLS**
Plate shaker, capable of shaking plates at a minimum of 900 rpm	88880023
Adjustable micropipettors	**MLS**
Multi-channel micropipettors	**MLS**
Reagents	
Ethanol, 96–100% (molecular biology grade)	**MLS**
Nuclease-free water	AM9932
Phosphate buffered saline (PBS (1X), pH 7.4)	10010023
Materials	
MicroAmp™ Clear Adhesive Film	4306311
4N6FLOQSwabs™, regular tip	4473979

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 2.0 Kit web page, scroll down to the **Product Literature** section.
2. Click **MagMAX_Ultra2_Direct_v2_FLEX** 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 the `SampleFile_384-Well Plate_192-Format.xlsx`.

Consider including 1 no-template control (using Nuclease-free water) to be run with test samples on the OpenArray™ plate.

Tool	Obtain from	Description
`SampleFile_384-Well Plate_192-Format.xlsx`	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.

Isolate DNA from buccal swabs

This section contains brief procedures. For detailed information, see *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.

General guidelines

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Precipitates and high viscosity can occur if Enhancer Solution and Binding Solution are stored when room temperature is too cold. If this occurs, warm them at 37°C and gently mix to dissolve precipitates and reduce viscosity. Avoid creating bubbles.
- Yellowing of the Binding and Wash I Solution is normal and will not affect buffer performance
- 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 using a plate shaker other than the recommended shaker, verify that:
  a. The plate fits securely on the plate shaker.
  b. The recommended speeds are compatible with the 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 10% overage.
- *(Optional)* To prevent evaporation and contamination, cover the prepared processing plates with paraffin film or MicroAmp™ Clear Adhesive Film until they are loaded into the instrument.

Before first use of the kit

Prepare Wash II Solution: Make 80% ethanol from 100% absolute ethanol and Nuclease-Free Water.

Before each use of the kit

Vortex DNA Binding Beads to fully resuspend the beads before each use.

Prepare samples and digest with Proteinase K

Equilibrate buccal swabs to room temperature, before performing isolation, to maximize DNA recovery.

1. Place one swab, swab-head down, into a deep-well plate.

   Note: When a higher concentration of DNA is required, process two swabs in one well and proceed with the isolation as indicated.

2. Break enough of the stick off the swab so that the swab sits in the well without protruding.

   The recommended swabs have an easy break point, below the swab, that appears as a slight indentation in the stick portion of the swab.

3. Prepare sufficient Proteinase K Mix according to the following table, then gently invert or pipet up and down several times to thoroughly mix components.

Component [1]	Volume per well	Volume per plate[2] (96 samples)
Enhancer Solution	40 µL	4.22 mL
PBS	400 µL	42.24 mL
Proteinase K	40 µL	4.22 mL
Total volume	**480 µL**	**50.68 mL**

[1] Pipet the components in the order they are listed in the table.
[2] Volumes include 10% overage.

IMPORTANT! Only make enough Mix for immediate use. Mix is not stable for prolonged periods and will result in a reduction of DNA yield.

4. Add 480 µL of the Proteinase K Mix to each well containing a swab.
   Be careful to avoid touching the pipette tip to the swab when pipetting the Proteinase K Mix into the sample wells.

5. Seal the plate with the clear adhesive film, then shake the sealed plate at 900 rpm for 5 minutes.

6. Take the plate off the plate shaker, then immediately incubate at 65°C for ≥20 minutes.

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

During the incubation, proceed to “Set up the instrument” on page 36 for automated purification using the KingFisher™ Flex Magnetic Particle Processor.

Set up the instrument

1. Ensure that the instrument is set up for processing with the proper magnetic head (96 deep-well) for your application.

2. Ensure that the proper heat block (96 well standard, not deep-well) is installed for your application.

3. Ensure that the proper program (**MagMAX_Ultra2_Direct__v2_FLEX**) has been downloaded from the product page and loaded onto the instrument.

Set up the processing plates

During the incubation at 65°C, set up the Wash, Elution, and Tip Comb Plates outside the instrument according to the following table.

Plate ID	Plate position	Plate type	Reagent	Volume per well
Wash I Solution Plate	2	Deep Well	Wash I Solution	1,000 µL
Wash II Solution Plate 1	3	Deep Well	Wash II Solution	1,000 µL
Wash II Solution Plate 2	4	Deep Well	Wash II Solution	500 µL
Elution Plate	5	Standard	Elution Solution	50 µL
Tip Comb	6	Place a 96 Deep-well Tip Comb in a Standard Plate		

Note: The plates will be loaded onto the instrument immediately after the Sample Plate has been prepared.

Bind the gDNA

1. Prepare the DNA Binding Bead Mix according to the following table.

Component	Volume per well	Volume per plate[1] (96 samples)
Binding Solution	400 µL	42.24 mL
DNA Binding Beads	40 µL	4.22 mL
Total volume	**440 µL**	**46.46 mL**

[1] Volumes include 10% overage.

2. At the end of Proteinase K digestion, shake the sealed plate at 900 rpm for 2 minutes.

3. Transfer the lysates to the corresponding wells of a new deep-well plate (this will be called the Sample Plate), then discard the buccal swabs.

To remove buccal swab from the lysate	Procedure
Transfer the lysate to a new plate.	1. Set a multi-channel pipettor to 480 µL and transfer one row at a time. 2. Ensure each well contains 420–480 µL after transfer.

4. Add 440 µL of DNA Binding Bead Mix to each sample.

Note: Remix Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. Mixture is viscous, pipet slowly to ensure that the correct amount is added.

IMPORTANT! Avoid creating bubbles during mixing and aliquoting.

5. Immediately process the plates on the KingFisher™ Flex Magnetic Particle Processor 96DW.

Wash and elute the gDNA

1. Select the program **MagMAX_Ultra2_Direct_v2_FLEX** on the instrument.
2. Start the run, then load the prepared plates into position when prompted by the instrument.
3. At the end of the run, immediately remove the plates from the instrument.
4. Transfer the eluate to the final tube/plate of choice for final storage.

   Note: If preferred, the elution plate may be used for final storage of the DNA.

The purified DNA is ready for immediate use. Alternatively, store the plate at –20°C for long-term storage.

Isolate DNA from whole blood

This section contains brief procedures. For detailed information, see *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 –80°C and –20°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. We recommend a new MicroAmp™ Clear Adhesive Film for each step of the procedure.
- If you use a plate shaker other than the recommended shaker, confirm the following items:
  - 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 Wash II Solution: Make 80% ethanol from 100% absolute ethanol and Nuclease-Free Water.

Before each use of the kit

Vortex DNA Binding Beads to fully resuspend the beads before each use.

Set up the instrument

1. Ensure that the instrument is set up for processing with the proper magnetic head (96 deep-well) for your application.
2. Ensure that the proper heat block (96 deep-well, not standard) is installed for your application.
3. Ensure that the proper program (**MagMAX_Ultra2_200µL_v2_FLEX** or **MagMAX_Ultra2_400µL_v2_FLEX**) has been downloaded from the product page and loaded onto the instrument.

Set up the processing plates

Set up the Wash, Elution, and Tip Comb Plates outside the instrument according to the following table.

Plate ID	Plate position	Plate type	Reagent	Volume per well
50–200 µL whole blood input				
Wash I SolutionPlate	2	Deep Well	Wash I Solution	500 µL
Wash II Solution Plate 1	3	Deep Well	Wash II Solution	500 µL
Wash II Solution Plate 2	4	Deep Well	Wash II Solution	500 µL
Elution Plate	5	Deep Well	Elution Solution	50–100 µL
Tip Comb	6	Place a 96 Deep-well Tip Comb in a Standard Plate		
200–400 µL whole blood input				
Wash I SolutionPlate	2	Deep Well	Wash I Solution	1,000 µL
Wash II Solution Plate 1	3	Deep Well	Wash II Solution	1,000 µL
Wash II Solution Plate 2	4	Deep Well	Wash II Solution	500 µL
Elution Plate	5	Deep Well	Elution Solution	50–100 µL
Tip Comb	6	Place a 96 Deep-well Tip Comb in a Standard Plate		

Note: The plates will be loaded onto the instrument immediately after the Sample Plate has been prepared in the next section.

Prepare Sample Plate and digest with Proteinase K

1. Transfer appropriate amount (according to the following table) of Enhancer Solution first, then sample, and lastly Proteinase K, to the appropriate wells of a deep-well plate. This will be the Sample Plate.

Enhancer Solution (µL)	Sample Volume (µL)	Proteinase K (µL)
5	50	5
10	100	10
20	200	20
30	300	30
40	400	40

Note:

- Do not pre-mix the Enhancer Solution and Proteinase K.
- Do not change the order of pipetting.
- Once all components are added, proceed immediately to the instrument processing. There is no need for manual mixing beforehand.

2. Select the program on the instrument according to the following table.

Whole blood input volume	Program
50–200 µL	**MagMAX_Ultra2_200µL_v2_FLEX**
200–400 µL	**MagMAX_Ultra2_400µL_v2_FLEX**

3. Start the run, and load the prepared plates into position when prompted by the instrument.

   During this on board Proteinase K sample digestion (~20 minutes) prepare the DNA Binding Bead Mix.

Purify the gDNA

1. Prepare DNA Binding Bead Mix according to the following table.

Component	Volume per well	Volume per plate[1]
50–200 µL whole blood input (96 samples per plate)		
Binding Solution	200 µL	21.12 mL
DNA Binding Beads	20 µL	2.11 mL
Total volume	**220 µL**	**23.23 mL**
200–400 µL whole blood input (96 samples per plate)		
Binding Solution	400 µL	42.24 mL

(continued)

Component	Volume per well	Volume per plate[1]
DNA Binding Beads	40 µL	4.22 mL
Total volume	**440 µL**	**46.46 mL**

[1] Volumes include 10% overage.

2. When instructed by the instrument (~20 minutes after the run has started), remove the Sample Plate and add DNA Binding Bead Mix to each sample, according to the following table.

For whole blood input volume	Add DNA Binding Bead Mix
50–200 µL	220 µL
200–400 µL	440 µL

Note: Remix Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. Mixture is viscous, pipet slowly to ensure that the correct amount is added.

IMPORTANT! Avoid creating bubbles during mixing and aliquoting.

3. Immediately place the plate back onto the KingFisher™ Flex and follow the prompts on the instrument to allow the sample processing to proceed.

4. At the end of the run, immediately remove the plate from the instrument and transfer the eluate to the final tube/plate of choice for final storage.

The purified DNA is ready for immediate use. Alternatively, store the plate at –20°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”.
   - **UV absorbance measurements**—Use a NanoDrop™ or other comparable instrument. The A_{260}/A_{280} ratio of high-quality gDNA is 1.8–2.0.
   - **Fluorometric analysis**—Use a Qubit™ dsDNA BR or HS Assay Kit.

2. Prepare a 50 ng/uL 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.

   (# of assays) × (Vol. of 5 ng/µL DNA per 10 µL reaction) × (# of replicates) × 120% (for dead volume)

   For example, if you are running 5 copy number assays, prepare at least:

   (5 Assays) × (2 µL) × (4 replicates) x 120%

   = 48 µL 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.

4 Prepare and run OpenArray™ PGx SNP genotyping experiments

This chapter contains brief procedures. For detailed procedures, see the following documents:

Document	Pub. No.
QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Experiments User Guide	4470935
OpenArray™ Sample Tracker Software Quick Reference, for OpenArray™ AccuFill™ Software v1.2	4460657
OpenArray™ AccuFill™ System User Guide, for OpenArray™ AccuFill™ Software v1.2	4456986
QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide, for OpenArray™ AccuFill™ Software v2.0	MAN0025669
OpenArray™ SNP Genotyping Experiments	MAN0014351
TaqMan™ OpenArray™ Genotyping Troubleshooting Guide	MAN0011115
TaqMan™ Genotyper Software Getting Started Guide	4448637

Workflow

Option 1: OpenArray™ AccuFill™ Software v1.2

Generate 384-well sample plate layouts in the OpenArray™ Sample Tracker Software
(page 53)

▼

Set up the PCR reactions in an OpenArray™ 384-well Sample Plate
(page 54)

▼

Set up the OpenArray™ AccuFill™ Instrument and the OpenArray™ AccuFill™ Software
(page 55)

▼

Transfer reactions to the OpenArray™ Plate using the OpenArray™ AccuFill™ Instrument
(page 56)

▼

Seal and run the OpenArray™ Plates
(page 67)

Option 2: OpenArray™ AccuFill™ Software v2.0

Download SPF files
(page 58)

▼

Set up the PCR reactions in an OpenArray™ 384-well Sample Plate
(page 59)

▼

Set up and start the run

▼

Remove the OpenArray™ Plate from the OpenArray™ AccuFill™ Instrument
(page 64)

▼

Seal and run the OpenArray™ Plates
(page 67)

Required materials for the OpenArray™ Plate workflow

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Instruments, software, and equipment	
OpenArray™ Sample Tracker Software (Not required for OpenArray™ AccuFill™ Software v2.0)	—[1]
QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0	A24945
QuantStudio™ 12K Flex Real–Time PCR System with OpenArray™ block (QuantStudio™ 12K Flex OpenArray™ AccuFill™ System)	4471090
Centrifuge, capable of spinning sample plates at 1,200 × *g*	MLS
Plates and accessories	
OpenArray™ 384-well Sample Plates, black *or* OpenArray™ 384-well Sample Plates, clear	4482221 4406947
(Optional) Biomek™ Seal and Sample Foil Lids (for pre-plating step)	Beckman Coulter™ 538619
OpenArray™ AccuFill™ System Tips	4458107
QuantStudio™ 12K Flex OpenArray™ Accessories Kit[2]	4469576
Forceps	MLS
A sharp edge, blade, or scalpel (to cut the adhesive foil)	MLS
A fine-tip silver or gold marker for black OpenArray™ 384-well Sample Plates A fine-tip black marker for clear OpenArray™ 384-well Sample Plates	MLS
Reagents	
Genomic DNA	See Chapter 3 on page 32
OpenArray™ Plates with PGx assays	4488847
TaqMan™ OpenArray™ Genotyping Master Mix	4404846

(continued)

Item	Source
100% molecular grade ethanol	MLS
Nuclease-free water	4387936

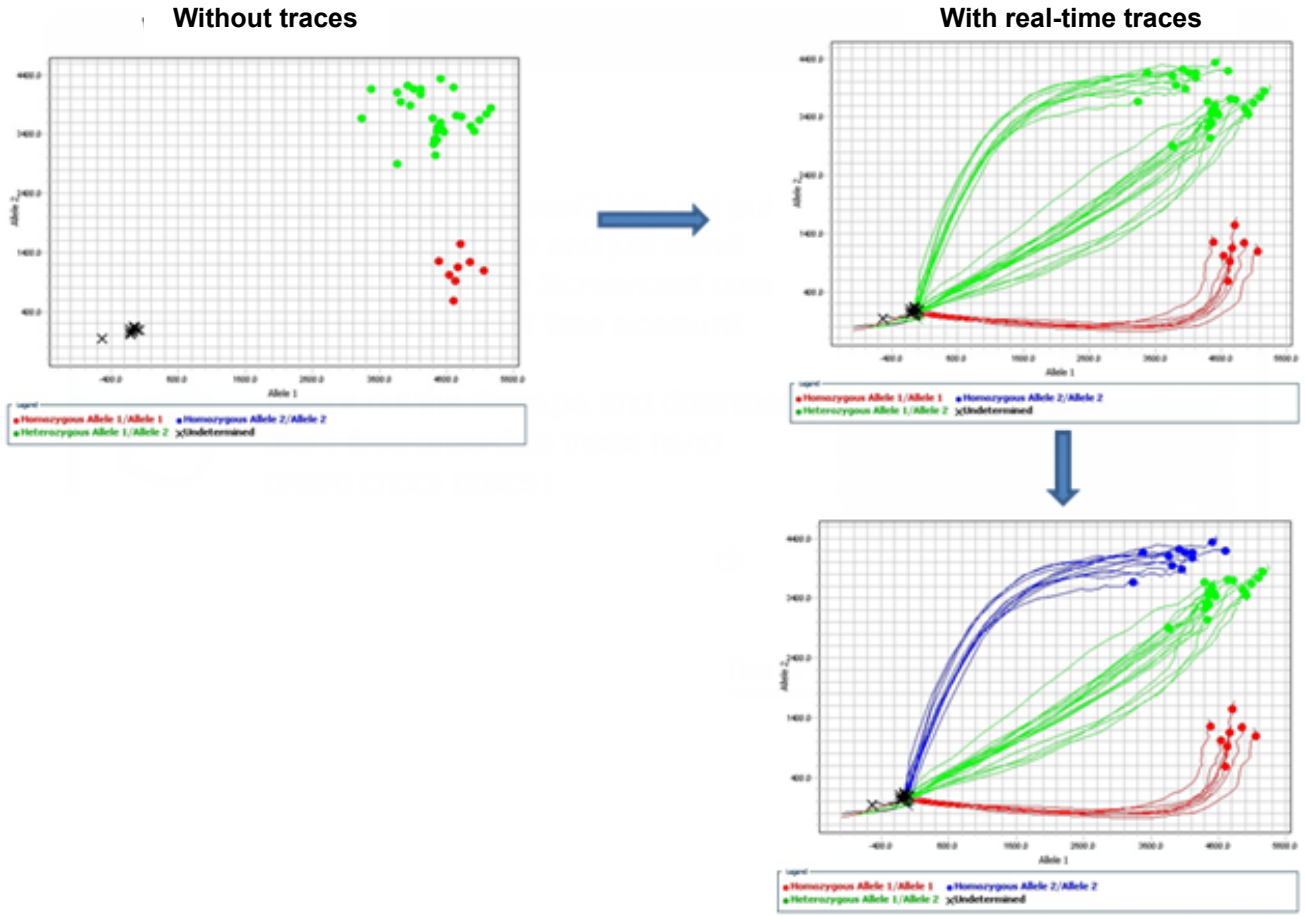
[1] Included with the QuantStudio™ 12K Flex Software.

[2] Each kit contains the items needed to assemble up to 10 plates: 12 lids and plugs, 12 immersion fluid syringes, and 2 carriers. Each custom OpenArray™ Plate order is shipped with accessories kits.

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.

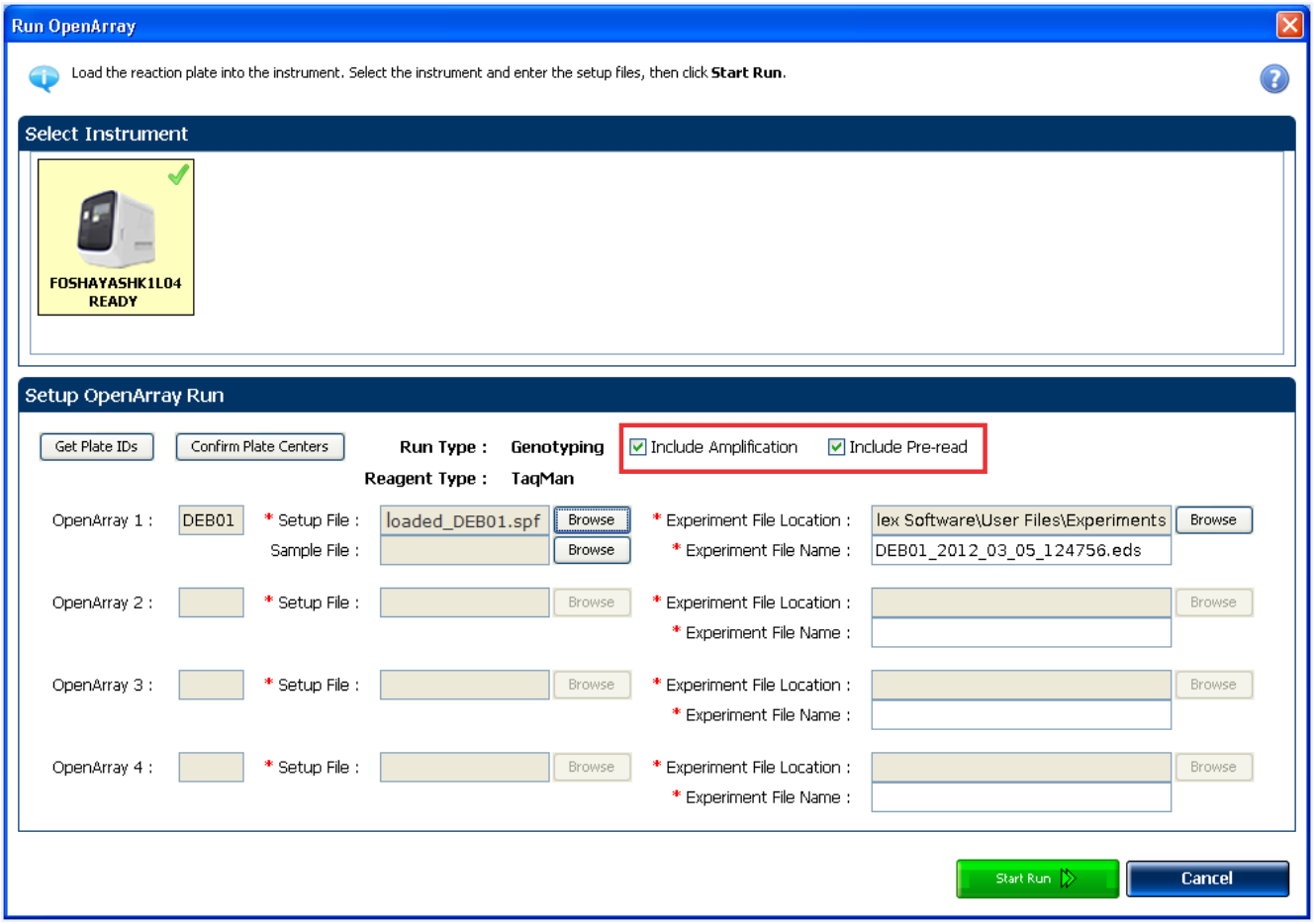
The following example shows how calls based on end-point reads could be incorrect when the DNA concentration is higher than the recommended amount.



Left: Calls based on end-point reads could be incorrect. Right: Real-time genotyping graphs used for making accurate calls when using samples with very high DNA concentration. Red: homozygous Allele 1/Allele 1. Blue: homozygous Allele 2/Allele 2. Green: heterozygous Allele 1/Allele 2.

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. 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).

Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v1.2

For required materials, see “Required materials for the OpenArray™ Plate workflow” on page 47.

For instructions for OpenArray™ AccuFill™ Software v2.0, see “Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v2.0” on page 58.

This chapter contains brief procedures. For detailed procedures, see the following documentation.

Document	Pub. No.
QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Experiments User Guide	4470935
OpenArray™ Sample Tracker Software Quick Reference	4460657
OpenArray™ AccuFill™ System User Guide	4456986

One-time procedures

Set up default folders and software preferences

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

Set up the default file locations and preferences before using the QuantStudio™ 12K Flex OpenArray™ AccuFill™ System for the first time.

1. Create the following four folders in a convenient location on the same computer drive as the OpenArray™ AccuFill™ Software:
   - `SPF Files`
   - `Sample Tracker 96-well Input`
   - `Sample Tracker 384-well CSV Files`
   - `Loaded SPF Files`
2. *(Optional)* Copy a template file into the OpenArray™ Sample Tracker Software folder.
   - Navigate to this folder on your computer: `<…>\Program Files (x86)\Applied Biosystems\OpenArray Sample Tracker\examples`, where <…> is the drive.
   - Copy the `96-Well Sample Plate 1.csv` template file, which is provided with the OpenArray™ Sample Tracker Software.
   - Paste the template file into the `Sample Tracker 96-well Input` folder.

3. In the 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** tip spacing
Import Data Directory	**Sample Tracker 96-well Input**
Export Data Directory	**Sample Tracker 384-well CSV Files**

4. In the OpenArray™ AccuFill™ Software, select **Instrument** ▸ **Edit Preferences** ▸ **Require Sample Integration**, then select the folders indicated in this table:

OpenArray™ AccuFill™ Software folder	Default folder	Folder contents
`OpenArray Plate File Input Folder`	`SPF Files`	SPF files for the OpenArray™ Plates, with 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 generated during processing with the OpenArray™ AccuFill™ Software.

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

Note: If the QuantStudio™ 12K Flex Software is not on the same computer as the OpenArray™ AccuFill™ Software, transfer the loaded SPF files to the computer running the QuantStudio™ 12K Flex 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 51.

To download SPF files for custom OpenArray™ Plates, you need the **Lot#** and the **Serial#** from the packaging of each OpenArray™ Plate.

1. Go to **thermofisher.com/OA-platefiles**.
2. From the **Select Your Product** dropdown list, select **TaqMan™ OpenArray™ Custom Gene Expression/Genotyping Plates**.
3. Select the desired option for downloading either only the SPF files or both the SPF files and the AIF files.

4. Enter the **Lot#** and the **Serial#**, then click **Submit**.

   Note: The **Serial#** is case-sensitive.

5. 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.

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

Before generating 384-well sample plate layouts, see “One-time procedures” on page 51 to complete the following tasks:

- Set up optimized folder locations and software preferences.
- Download the SPF files for the OpenArray™ Plates into the SPF Files folder.

1. Using a spreadsheet program, create a 96-well sample CSV file.
   a. Navigate to the following folder, then open the `96-Well Sample Plate 1.csv` template that is provided with the OpenArray™ Sample Tracker Software.

      `<…>\Program Files (x86)\Applied Biosystems\OpenArray Sample Tracker\examples`, where <…> is the drive.

   b. **Save As** the template as a new 96-well sample CSV file. Save your 96-well sample CSV file in the **Sample Tracker 96-well Input** folder.

   c. Enter or copy the sample names into your 96-well sample CSV file.
2. Open the OpenArray™ Sample Tracker Software.
3. In the **Properties** screen, select **Genotyping** for **Experiment Type**, then select the appropriate settings for **OpenArray™ Plate** and **Pipettor**.
4. In the **Samples** screen, click **Import**, then select and import your 96-well sample CSV file that you created in step 1.
5. In the **Sample Mapping** screen, confirm that the samples for a single OpenArray™ Plate are assigned to one color.

   Note: If necessary, correct the **OpenArray™ Plate** and **Pipettor** settings in the **Properties** screen.

6. In the **Sample Mapping** screen, click the **384-Well Plate** tab, then click **Export** ▸ **Export *.csv**.
7. Select **384-Well Plate (for AccuFill)**, enter a file name, 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 PCR reactions in an OpenArray™ 384-well Sample Plate

IMPORTANT! The 4 × 12 areas of the OpenArray™ 384-well Sample Plate being filled must match the areas designated in the OpenArray™ Sample Tracker Software for that set of samples.

1. Remove an OpenArray™ Plate from the freezer and set it aside. 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 OpenArray™ 384-well Sample Plate created in this section.

2. Gently swirl the contents of the TaqMan™ OpenArray™ Genotyping Master Mix to thoroughly mix. Do not invert the bottle.

3. 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.

Component	OpenArray™ Plate Format		
	16	32	64+
	Volume per well		
TaqMan™ OpenArray™ Genotyping Master Mix	1.5 µL	2.0 µL	2.5 µL
One of the following: • 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**

4. Thoroughly mix each PCR reaction by pipetting up and down or by using the "mix" function on a multi-channel pipette. Alternatively, vortex the OpenArray™ 384-well Sample Plate for 10–15 seconds after sealing with aluminum foil in step 5.

5. Seal the OpenArray™ 384-well Sample Plate with an aluminum foil seal, remove the foil flap, then mark the edges of the filled 4 × 12 area with a pen.

6. Vortex the plate for 20–30 seconds, then centrifuge the plate at 1,200 × *g* for 1 minute.

7. Score the foil along the lines that were marked before centrifuging.

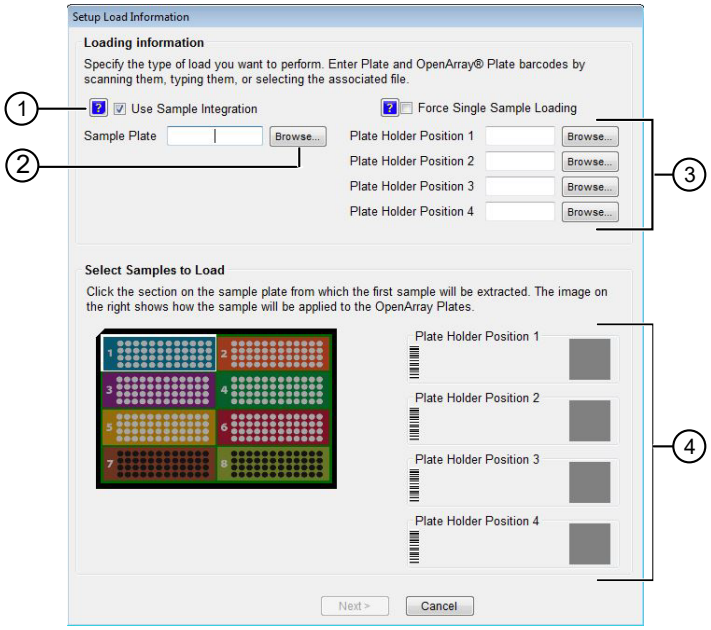
   Do not remove the foil from the scored area at this time.

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 53 with a corrected 96-well sample CSV file.

Set up the OpenArray™ AccuFill™ Instrument and the OpenArray™ AccuFill™ Software

IMPORTANT! Do not use OpenArray™ AccuFill™ System Tips that exceed the expiration date (shown on the outer box that contains the tip trays).

1. In the OpenArray™ AccuFill™ Software, click **Setup and Load**.
   The **Setup Load Information** window appears.



① **Use Sample Integration** checkbox; select to integrate SPF files and the 384-well sample plate CSV file.

② **Browse** button; click to locate and select the 384-well sample plate CSV file. The button is displayed only if **Use Sample Integration** is selected.

③ **Browse** buttons; click to locate and select the SPF files for the OpenArray™ Plates that will be placed in the corresponding **Plate Holder Position** on the deck of the OpenArray™ AccuFill™ Instrument. The buttons are displayed only if **Use Sample Integration** is selected.

④ **Plate Holder Position** corresponding to the position of the OpenArray™ Plate on the deck of the instrument.

2. Configure the **Loading Information** pane for sample integration using the 384-well sample plate CSV file and SPF files.
   a. In the **Loading Information** pane (top section of the window), ensure that the **Use Sample Integration** checkbox is selected.

   b. Click **Browse** to the right of the **Sample Plate** field, then select the 384-well sample plate CSV file that you generated with the OpenArray™ Sample Tracker Software in the Sample Tracker 384-well CSV Files folder.

   c. Click **Browse** to the right of the **Plate Holder Position** of the OpenArray™ Plate, then select the SPF file for the OpenArray™ Plate in the SPF Files folder.

3. In the **Select Samples to Load** pane (bottom section of the window), click the corresponding 4 × 12 area of the 384-well sample plate image, then click **Next**.
   The **Setup Deck** window is displayed.

4. In the OpenArray™ AccuFill™ Instrument, ensure that:
   - Tip boxes and tips are loaded as shown in the **Setup Deck** window.
   - The lids are removed from the tip boxes.
   - The waste bin in the instrument is emptied.

5. In the **Setup Deck** window, confirm that the deck is ready:
   - Select **The tips are configured as shown above**.
   - Select **The Waste Bin is empty**.

Transfer reactions to the OpenArray™ Plate using the OpenArray™ AccuFill™ Instrument

IMPORTANT! Ensure that the OpenArray™ Plate is thawed and that the entire plate is at room temperature.

1. Prepare the items needed to seal the loaded OpenArray™ Plate (next section).

   Note: The OpenArray™ Plate must be sealed promptly after being loaded with the reactions, as described here.

   - Ensure that the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0 is ready.
   - Gather and remove from their packaging the following: an OpenArray™ Lid, plug, syringe with OpenArray™ Immersion Fluid, and syringe tip.
   - Attach the syringe tip to the syringe, carefully push some of the fluid through the tip to remove air bubbles, then lay the syringe aside.

2. Load the OpenArray™ Plate and the OpenArray™ 384-well Sample Plate into the OpenArray™ AccuFill™ Instrument.
   - **OpenArray™ Plate**—Remove the plate from its sleeve, then place the plate in the appropriate plate holder position in the instrument.
     Ensure that the barcode on the OpenArray™ Plate is facing left and the serial number is facing right.
   - **OpenArray™ 384-well Sample Plate**—Place the 384-well sample plate onto the deck of the instrument, then use forceps to peel the foil from the filled area of the plate.

3. Close the door of the instrument.

4. In the OpenArray™ AccuFill™ Software **Setup Deck** window, select the following confirmations:
   - **The OpenArray Plate is in the Plate Holder**
   - **Remove foil from the highlighted section of the Sample Plate**

5. Click **Load**.

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.
   See “Seal the OpenArray™ Plate” on page 67.

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

Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v2.0

For required materials, see “Required materials for the OpenArray™ Plate workflow” on page 47.

For instructions for OpenArray™ AccuFill™ Software v1.2, see “Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v1.2” on page 51.

This chapter describes the full run workflow. For other workflow options, see the following documentation.

This chapter contains brief procedures. For detailed procedures, see the following documentation.

Document	Pub. No.
QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Experiments User Guide	4470935
QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide	MAN0025669
OpenArray™ AccuFill™ Software v2.0 Quick Run Workflow Without Sample Information Quick Reference	MAN0025835
OpenArray™ AccuFill™ Software v2.0 Full Run Workflow Quick Reference	MAN0025836

Download SPF files

The computer with the OpenArray™ AccuFill™ Software v2.0 must be connected to the internet.

1. In the **TPF/SPF** screen, select the **Download** radio button.
2. Select the product.
   - **TaqMan OpenArray Custom**
   - **TaqMan OpenArray Inventoried**
3. Enter the following information.

Product	Information
TaqMan OpenArray Custom	a. Enter the ***Lot number*** or ***Batch number***. b. Enter one ***Serial number*** from the lot. **Note:** Only one serial number is required. The serial number is used to confirm the lot number or batch number. All of the files in the lot or batch are downloaded.
TaqMan OpenArray Inventoried	Enter the list of ***Serial numbers*** or ***Barcodes***. Separate more than one serial number or barcode with a comma or a line break. **Note:** The serial number or barcode entered corresponds to the file that is downloaded. Enter a serial number or barcode for each file to download.

Note: The fields that are displayed depend on the product selected in step 2.

4. Click **Download**.

The location of the files is displayed at the top of the screen. The location of the downloaded files is set in the **Preferences** menu, in the **OpenArray plate file folder** field. See *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669) for more information about setting the preferences.

The files are in a compressed ZIP folder.

Click **Open folder** to access the files or click ✖ **(Close)** to close the message.

Extract the files from the compressed ZIP folder.

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

IMPORTANT! The 4 × 12 areas of the OpenArray™ 384-well Sample Plate being filled must match the areas designated in the OpenArray™ AccuFill™ Software for that set of samples.

1. Remove an OpenArray™ Plate from the freezer and set it aside. 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 OpenArray™ 384-well Sample Plate created in this section.

2. Gently swirl the contents of the TaqMan™ OpenArray™ Genotyping Master Mix to thoroughly mix. Do not invert the bottle.

3. 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.

Component	OpenArray™ Plate Format		
	16	32	64+
	Volume per well		
TaqMan™ OpenArray™ Genotyping Master Mix	1.5 µL	2.0 µL	2.5 µL
One of the following: • 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**

4. Thoroughly mix each PCR reaction by pipetting up and down or by using the "mix" function on a multi-channel pipette. Alternatively, vortex the OpenArray™ 384-well Sample Plate for 10–15 seconds after sealing with aluminum foil in step 5.

5. Seal the OpenArray™ 384-well Sample Plate with an aluminum foil seal, remove the foil flap, then mark the edges of the filled 4 × 12 area with a pen.

6. Vortex the plate for 20–30 seconds, then centrifuge the plate at 1,200 × *g* for 1 minute.

7. Score the foil along the lines that were marked before centrifuging.

   Do not remove the foil from the scored area at this time.

If you make a sample layout error, it is possible to correct this in the OpenArray™ AccuFill™ Software using the plate rotation feature. For more information, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

Set up and start the run

Before you begin—full run workflow

- Prepare samples in a 384-well plate (see “Set up the PCR reactions in an OpenArray™ 384-well Sample Plate” on page 59).
- Place the sample plate in the sample plate holder on the instrument deck, with the notch to the left.

  Do not stack sample plates.
- Load the tip boxes, then remove the tip box covers.

  Do not stack the tip boxes.
- Place the OpenArray™ Plates in the plate holders.
- Clear the instrument deck, empty and replace the waste bin, then close the instrument door.
- Allow the instrument to perform a self-test if the run is being started after the software is launched.
- Prepare the materials in the QuantStudio™ 12K Flex OpenArray™ Accessories Kit

  These materials are used to seal the OpenArray™ Plates.

  IMPORTANT! OpenArray™ Plates must be sealed immediately after loading.

Configure the experiment design for the full run workflow

A SPF file *is* required for this workflow.

Navigate to the **Full Run** screen.

1. In the **Configure design** pane, in the **Experiment type** section, select **Genotyping**.
2. In the **Plate format** section, select a format.

3. If the **Pipettor** section is displayed, select a type of pipette.
   - **Fixed**
   - **Adjustable**
4. In the **Add your OpenArray Plate serial numbers** section, click **Choose File**, navigate to the location of the SPF file, then select the file.
   Repeat for each SPF file.
5. In the **Add your sample plates - optional** section, click **Choose File**, navigate to the location of the CSV file, then select the file.
   The format of the sample plate file is validated. For information about the required format, see the *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).
   The name of the file is displayed in the **Select file** field.
6. Repeat step 5 for each CSV file.
7. Click **Next**.

The **Map plates** pane is displayed.

Proceed to "Add or edit sample names" on page 61.

Add or edit sample names

If needed, navigate to the **Map plates** pane in the **Full Run** screen.

If a sample plate file was imported, the sample names are displayed. The sample plate layout defined in the sample plate file can be edited.

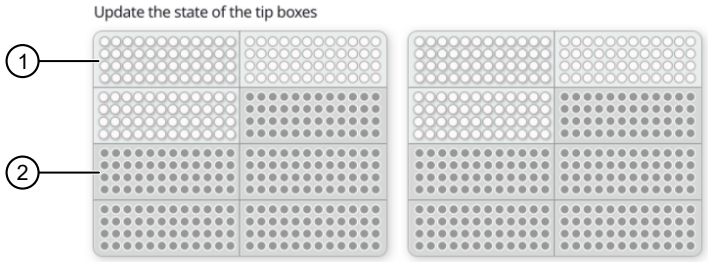
If the sample plate file was not imported, the samples must be added manually.

1. Add or edit the sample name.
2. Click **Next**.

Proceed to "Verify the run setup and start the run" on page 61.

Verify the run setup and start the run

1. Click each tip box section so that the status on the **Verify and start run** pane matches the physical tip box in the instrument.
   We recommend starting the run with full tip boxes.
   The instrument does not start the run if there are not enough tips on the deck.



① Section of the tip box that is full
② Section of the tip box that is empty

2. *(Optional)* Click **Auto-fill tip boxes**.

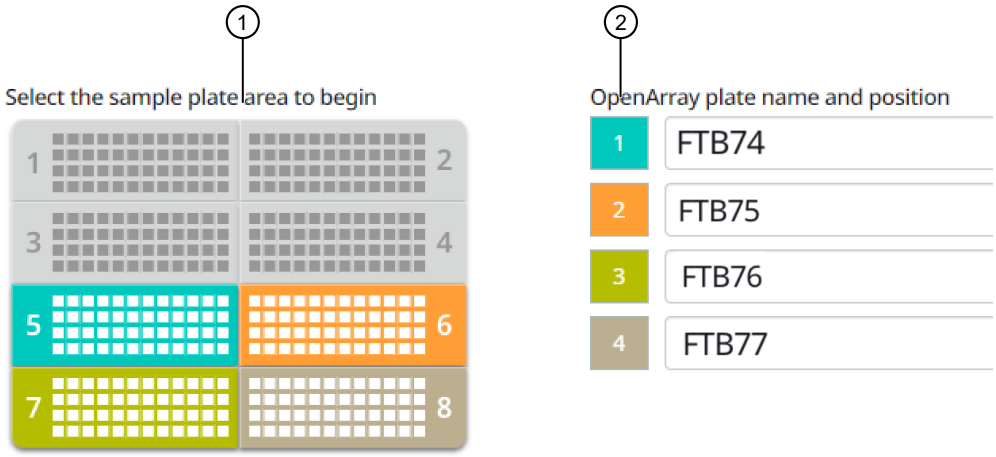
   The status of all section of the tips boxes is set to full.

3. Select the first section of the sample plate that will be used to fill the OpenArray™ Plate.

   Select the first section of the sample plate if multiple OpenArray™ Plates are filled during a run. The software selects the total number of sections that correspond with the total number of OpenArray™ Plates.

   In the following example, section 5 was selected. The group of sections 5, 6, 7, and 8 is highlighted by the software because four OpenArray™ Plates are being filled.

   The position box displays the color that corresponds to the section of the sample plate.



① Sample plate section (section 5, 6, 7, and 8 are highlighted)
② Corresponding OpenArray™ Plates

4. Remove the foil from the appropriate sections of the sample plate, then click the checkbox to confirm.

   Remove the foil only from the sections of the sample plate that are used to load a single OpenArray™ Plate.

   Note: Do not remove the foil from all the sections of the sample plate at once.

5. Close the instrument door.

6. Click **Start Run**.

   The run does not begin under any of the following conditions:

   - The waste bin is not in position
   - The sample plate is not in position
   - The OpenArray™ Plates are not in position
   - There are more OpenArray™ Plates on the instrument deck than are defined in the experiment setup

The **Deck** screen is displayed.

For a description of the run progress, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

IMPORTANT! Each OpenArray™ Plate must be prepared for PCR immediately after it is filled (see “Remove the OpenArray™ Plate from the OpenArray™ AccuFill™ Instrument” on page 64).

Remove the OpenArray™ Plate from the OpenArray™ AccuFill™ Instrument

After an OpenArray™ Plate is filled, the **Remove plate and foil** dialog box is displayed (see Figure 1 on page 64).

Remove each OpenArray™ Plate *immediately* after it has been filled, even if the run was set up to fill multiple plates.

After the last OpenArray™ Plate in the run is filled, the **Remove plate** dialog box is displayed (see Figure 2 on page 65).

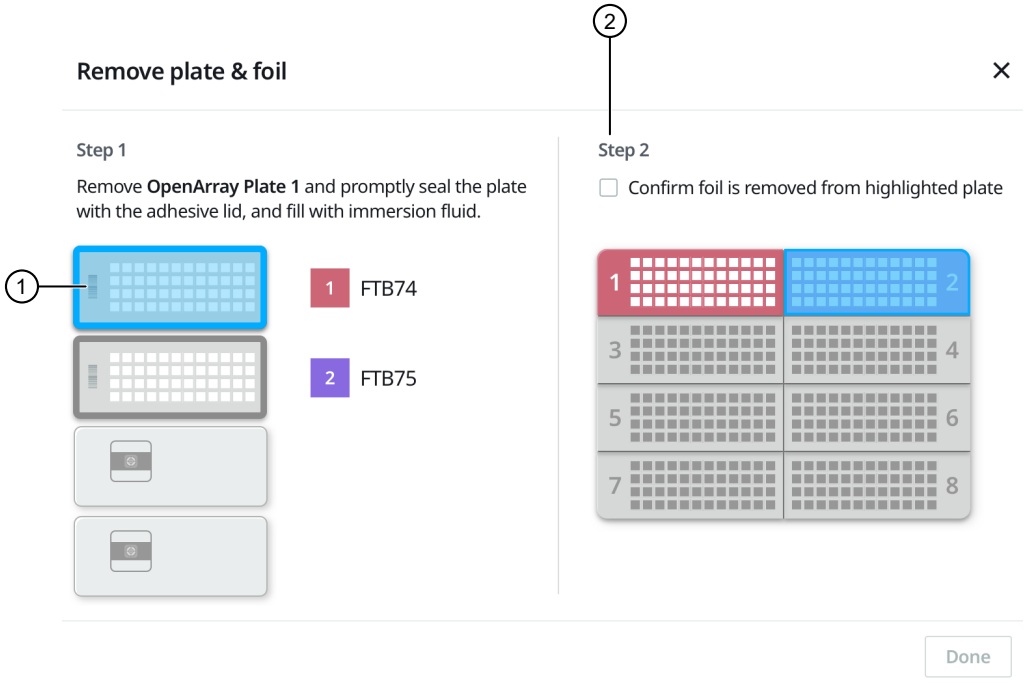


Figure 1 Remove plate and foil dialog box

① OpenArray™ Plate to remove from the instrument
② **Confirm foil is removed from highlighted plate section** checkbox

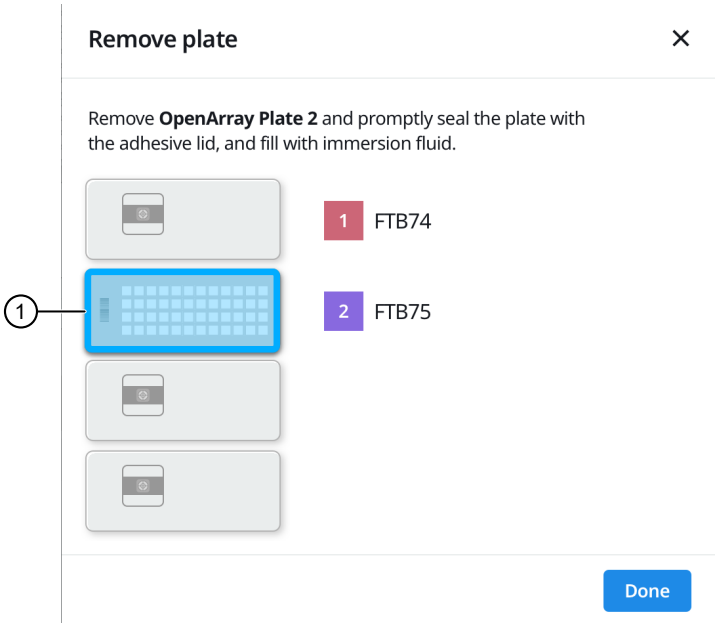


Figure 2 Remove plate dialog box

① OpenArray™ Plate to remove from the instrument

1. Open the instrument door and remove the OpenArray™ Plate that is indicated by the blue box in the dialog box.

   IMPORTANT! Remove the OpenArray™ Plate immediately, to avoid evaporation within the plate.

   One of the following dialog boxes is displayed:

   - The **Remove plate and foil** dialog box.
   - The **Remove plate** dialog box (after the last OpenArray™ Plate is filled).

2. Seal the case and fill the OpenArray™ Plate with immersion fluid.

   See “Seal the OpenArray™ Plate” on page 67.

3. *(For **Remove plate and foil** dialog box only)* Remove the foil seal from the next section of the sample plate, then select the checkbox to confirm that the foil is removed from the section of the plate that is highlighted.

   Note: Remove the foil only from the next section of the sample plate. Do not remove the foil from all sections of the sample plate.

4. Close the instrument door.

5. Click **Done**.

   The run does not proceed under any of the following conditions:

   - The waste bin is not in position
   - The sample plate is not in position
   - The OpenArray™ Plates are not in position
   - There are more OpenArray™ Plates on the instrument deck than are defined in the experiment setup

   The instrument will proceed to load the next OpenArray™ Plate.

6. Repeat step 1 to step 5 for each OpenArray™ Plate to be loaded.

After all of the OpenArray™ Plates have been loaded, the **Deck** screen displays **Run completed successfully. Empty the waste bin before performing another run.**

A loaded SPF is generated for each OpenArray™ Plate. The loaded SPF file corresponds to the original SPF file that was imported for the run. The files are exported to the folder that was designed in the **Preferences**.

Note: Some workflows might not generate a loaded SPF file. For more information about the workflows available for the OpenArray™ AccuFill™ Software v2.0, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

Seal and run the OpenArray™ Plates

Seal the OpenArray™ Plate

IMPORTANT! Throughout this procedure, handle the OpenArray™ Plate and the OpenArray™ Case only by the edges.

Note: The OpenArray™ Case consists of the sealed OpenArray™ Plate and the OpenArray™ Lid.

1. Place the newly loaded OpenArray™ Plate in the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0.

   Ensure that the barcode is facing left and the serial number is facing right.

2. From the OpenArray™ Lid, remove the clear protective film from the *inside* of the lid ① and the red adhesive-protective strip ② from around the edge of the lid.

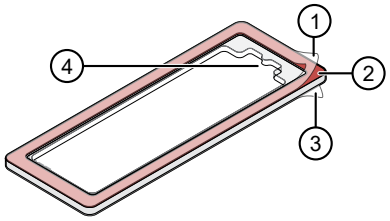
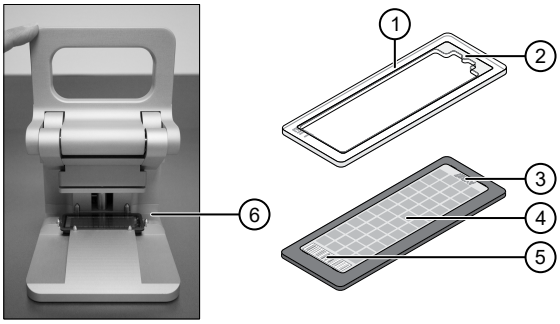


Figure 3 OpenArray™ Lid

① Protective film on inside of the lid (remove before *sealing*)
② Red adhesive-protective strip (remove before *sealing*)
③ Protective film on the outside of the lid (remove before *running*)
④ Notched end (align with serial number on plate)

3. Place the lid in the Plate Press using the alignment pins of the Plate Press for orientation.

IMPORTANT! The notched end of the case lid must be oriented towards the furthest back right-side of the Plate Press.



① OpenArray™ case lid
② Notched end of lid
③ Serial number of plate
④ OpenArray™ Plate
⑤ Barcode of plate
⑥ Alignment pins

4. Seat the lid on the OpenArray™ Plate with the lid adhesive against the plate.

5. Engage the press mechanism until the green flashing light changes to a steady green light (after 20 seconds).

   The status light turns solid green, indicating that the case is sealed.

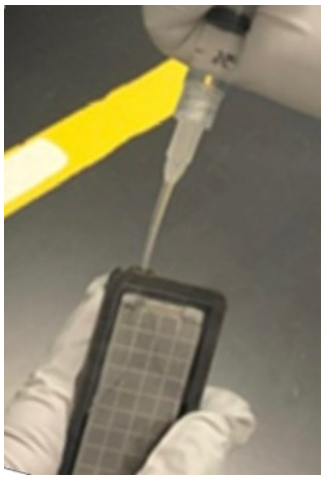
Note: Do not apply additional pressure onto the Plate Press during its actuation.

6. Disengage the press and carefully remove the OpenArray™ Case.

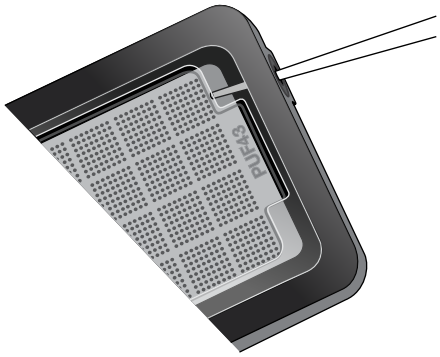
7. Prepare the immersion fluid. Remove the cap, insert the accompanying syringe tip, and prime the syringe by ejecting a small amount of immersion fluid onto a paper towel to ensure no air gap remains in the newly attached pipette tip.

IMPORTANT! If the syringe is not primed, the direct burst of air and fluid can negatively affect the assay(s) at the end of the array.

8. While holding the case upright by its edges at a 15–30 degree angle so that the port is at the highest point of the array, insert the prepared syringe tip into the port in the case.



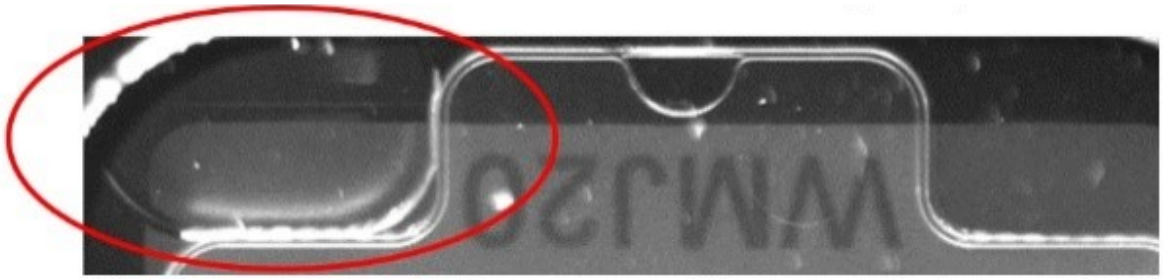
The syringe tip must be in front of the array when filling the case with immersion fluid.



9. Slowly inject the OpenArray™ Immersion Fluid until the case is filled, which should take about 10 seconds to fill. Minimize the creation of additional air bubbles when you dispense the fluid. Leave a small air bubble as shown below.

IMPORTANT! If injected too quickly, the fluid can flush out the samples that are suspended in the through-holes.

Overfilling the array and/or not leaving a small bubble may cause a leak during the PCR run.



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

    Note: Ensure that you are screwing the plug in at the same angle the case base is at. If it is off, it can cause the plug to break off prematurely.

    IMPORTANT! To avoid leaking of immersion fluid, hold the case *vertically* and rotate the plug slowly to avoid cross-threading.

    If the plug handle breaks off prematurely, use a Phillips #0 screwdriver to complete this step. Do not overtighten. If plastic or adhesive remains attached to the screw due to premature breakout of the plug handle, remove it with forceps prior to loading it into the instrument.

11. If needed, clean the case with the lint-free cloth included with the OpenArray™ Plate or a laboratory wipe that has been thoroughly sprayed with ethanol, then dry the case with a clean laboratory wipe.

Run the OpenArray™ Plate on the QuantStudio™ 12K Flex Real–Time PCR System

You can run up to four OpenArray™ Plates at one time on the QuantStudio™ 12K Flex Real–Time PCR System.

1. On the QuantStudio™ 12K Flex Real–Time PCR System touchscreen, touch  to extend the instrument tray arm.
2. Remove the clear protective film from the outside of the OpenArray™ case (sealed plate + lid).
3. Place the OpenArray™ case on the tray arm plate adapter.
   - Support the case from underneath the tray arm to prevent the case from slipping through the adapter.
   - Ensure that the plate barcode and serial number are facing the front of the instrument.
   - Ensure that the OpenArray™ Plate adapter A1 position is aligned with the instrument arm adapter A1 position.
4. Touch  to retract the instrument tray arm.
5. In the  **Home** screen of the QuantStudio™ 12K Flex Software, in the **Run** pane, click **OpenArray**.
6. In the **Select Instrument** pane, select your instrument.
7. Click **Get Plate IDs** to import the barcode of the OpenArray™ Plate.
   Once the OpenArray™ serial number appears, the loaded SPF file corresponding to the plate should appear in the **Setup File** field.
   If the SPF file does not appear, click **Browse**, then select the correct loaded SPF file from the **Loaded SPF** folder.
8. *(Optional)* Click **Browse** to change the **Experiment File Location**.
9. *(Optional)* Change the software-determined **Experiment File Name**.

10. Click **Start Run**.

    Note: The instrument pauses prior to the end of the run. Wait for the system to complete the run before opening the EDS file.

11. Transfer the EDS file from the instrument to an accessible location for analysis.

12. Check the QC images for loading issues or leaks.

Check the quality–control images

Check the quality–control (QC) images before analysis. Images can be viewed using ImageJ, an open–source software available from the NIH at **imagej.nih.gov/ig**.

1. In the QuantStudio™ 12K Flex Software **Export** screen, click **Browse**, then create a uniquely–named folder for the QC images export.

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

2. Click **Export QC Images** at the bottom of the screen.

3. View the following ROX™ image to check for loading quality issues:
   - POST-READ_CHANNEL_4.tiff

4. Check the following spotfinding images for leaks or other displaced sample issues.
   - s02_c001_t03_p0001_m1_x2_e1_cp#_spotfind.tiff
   - s02_c040_t03_p0001_m1_x2_e1_cp#_spotfind.tiff

   Note: The “cp#” in the image file name refers to array positions 1 through 4 within the instrument.

5. If a problem is found, view the following pre-run spotfinding image to determine whether the issue existed before cycling:
   - s00_c001_t01_p0001_m2_x3_e1_cp#_spotfind.tiff

6. View the following FAM™ images to check for fluorescent abnormalities and to confirm any problem seen in the spotfinding images:
   - STAGE2_CYCLE1_CHANNEL_1.tiff
   - STAGE2_CYCLE40_CHANNEL_1.tiff

7. Note any abnormalities found, as well as all other potentially relevant information related to the setup of the run.

5 Prepare and run OpenArray™ PGx SNP genotyping experiments using EDT files

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

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

Workflow

Option 1: OpenArray™ AccuFill™ Software v1.2

Download SPF files
(page 76)

Create an array-specific template (EDT) file
(page 76)

Generate OpenArray™ Plate layouts in the OpenArray™ Sample Tracker Software
(page 77)

Set up the PCR reactions in an OpenArray™ 384-well Sample Plate
(page 78)

Set up the OpenArray™ AccuFill™ Instrument and the OpenArray™ AccuFill™ Software
(page 79)

Transfer reactions to the OpenArray™ Plate using the OpenArray™ AccuFill™ Instrument
(page 80)

Seal and run the OpenArray™ Plates
(page 91)

Option 2: OpenArray™ AccuFill™ Software v2.0

Required materials for the OpenArray™ Plate workflow

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Instruments, software, and equipment	
OpenArray™ Sample Tracker Software (Not required for OpenArray™ AccuFill™ Software v2.0)	—[1]
QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0	A24945
QuantStudio™ 12K Flex Real–Time PCR System with OpenArray™ block (QuantStudio™ 12K Flex OpenArray™ AccuFill™ System)	4471090
Centrifuge, capable of spinning sample plates at 1,200 × *g*	MLS

(continued)

Item	Source
Plates and accessories	
OpenArray™ 384-well Sample Plates, black *or* OpenArray™ 384-well Sample Plates, clear	4482221 4406947
(Optional) Biomek™ Seal and Sample Foil Lids (for pre-plating step)	Beckman Coulter™ 538619
OpenArray™ AccuFill™ System Tips	4458107
QuantStudio™ 12K Flex OpenArray™ Accessories Kit[2]	4469576
Forceps	MLS
A sharp edge, blade, or scalpel (to cut the adhesive foil)	MLS
A fine-tip silver or gold marker for black OpenArray™ 384-well Sample Plates A fine-tip black marker for clear OpenArray™ 384-well Sample Plates	MLS
Reagents	
Genomic DNA	See page 32
OpenArray™ Plates with PGx assays	4488847
TaqMan™ OpenArray™ Genotyping Master Mix	4404846
100% molecular grade ethanol	MLS
Nuclease-free water	4387936

[1] Included with the QuantStudio™ 12K Flex Software.

[2] Each kit contains the items needed to assemble up to 10 plates: 12 lids and plugs, 12 immersion fluid syringes, and 2 carriers. Each custom OpenArray™ Plate order is shipped with accessories kits.

Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v1.2

For required materials, see “Required materials for the OpenArray™ Plate workflow” on page 74.

For instructions for OpenArray™ AccuFill™ Software v2.0, see “Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v2.0” on page 82.

This chapter contains brief procedures. For detailed procedures, see the following documentation.

Document	Pub. No.
QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Experiments User Guide	4470935
OpenArray™ Sample Tracker Software Quick Reference	4460657
OpenArray™ AccuFill™ System User Guide	4456986

Download SPF files

To download an SPF file for a custom OpenArray™ Plate, you need the **Lot#** and the **Serial#** from the packaging of the OpenArray™ Plate.

Note: To prepare an array-specific template EDT file, you only need to download an SPF file once. If there is a change to the plate layout, you will need to download a new SPF file, then create a new template.

1. Go to **thermofisher.com/OA-platefiles**.
2. From the **Select Your Product** dropdown list, select **TaqMan™ OpenArray™ Custom Gene Expression/Genotyping Plates**.
3. Select the desired option for downloading either only the SPF files, or downloading both the SPF files and the AIF files.
4. Enter the **Lot#** and the **Serial#**, then click **Submit**.

   Note: The **Serial#** is case-sensitive.
5. Save the SPF file to the desktop.

Create an array-specific template (EDT) file

1. In the **Home** screen of the QuantStudio™ 12K Flex Software, in the **Experiment** pane, click **Create From Template**.

   An **Open** window will appear.
2. In the **Look in** field, select **OpenArray**, click **Genotyping** (file name **Genotyping.edt)**, then click **Open**.

   A new experiment is created using the setup information from the template.

3. From the open experiment, click the **Targets** tab on the left pane, then click **Import** ▸ **Import Plate Setup**.
4. Click **Browse** to select the SPF plate file for the desired panel, then click **Select**.
5. Click **Start Import** ▸ **Yes** to confirm the import.
6. Click **Save As Template** to save the array-specific EDT file.
7. Click **Save**.

Generate OpenArray™ Plate layouts in the OpenArray™ Sample Tracker Software

1. Using a spreadsheet program, create a 96-well sample CSV file.

   CSV template files can be found at `<…>\Program Files (x86)\Applied Biosystems\OpenArray Sample Tracker\examples`, where <…> is the drive.

   a. Navigate to the following folder, then open the `96-Well Sample Plate 1.csv` template that is provided with the OpenArray™ Sample Tracker Software.

      `<…>\Program Files (x86)\Applied Biosystems\OpenArray Sample Tracker\examples`, where <…> is the drive.

   b. **Save As** the template as a new 96-well sample CSV file.

   c. Enter or copy the sample names into your 96-well sample CSV file.

2. Open the OpenArray™ Sample Tracker Software.
3. In the **Properties** screen, select **Genotyping** for **Experiment Type**, then select the appropriate settings for **OpenArray™ Plate** and **Pipettor**.
4. In the **Samples** screen, click  **Import**, then select and import your 96-well sample CSV file that you created in step 1.
5. In the **Sample Mapping** screen, confirm that the samples for a single OpenArray™ Plate are assigned to one color.

   Note: If necessary, correct the **OpenArray™ Plate** and **Pipettor** settings in the **Properties** screen.

6. In the **Sample Mapping** screen, click **Export** ▸ **Export *.csv**.

   Note: You can export up to four CSV files at once.

7. Select **OpenArray Plate (1, 2, 3, or 4) (for QuantStudio)**, enter a file name, then save the exported file(s).

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

IMPORTANT! The 4 × 12 areas of the OpenArray™ 384-well Sample Plate being filled must match the areas designated in the OpenArray™ Sample Tracker Software for that set of samples.

1. Remove an OpenArray™ Plate from the freezer and set it aside. 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 OpenArray™ 384-well Sample Plate created in this section.

2. If stored in the freezer, thaw your 96-well DNA sample plates (~15 minutes).

   Once thawed, vortex briefly, then centrifuge each plate at 1,000 × *g* for 1 minute to collect liquid at bottom of the wells.

3. Gently swirl the contents of the TaqMan™ OpenArray™ Genotyping Master Mix to thoroughly mix. Do not invert the bottle.

4. Using a new OpenArray™ 384-well Sample Plate, define the four quadrants that correspond to each 96-well DNA sample plate with a black marker.

5. 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.

Component	OpenArray™ Plate Format		
	16	32	64+
	Volume per well		
TaqMan™ OpenArray™ Genotyping Master Mix	1.5 µL	2.0 µL	2.5 µL
One of the following: • 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**

6. Thoroughly mix each PCR reaction by pipetting up and down or by using the "mix" function on a multi-channel pipette. Alternatively, vortex the OpenArray™ 384-well Sample Plate for 10–15 seconds after sealing with aluminum foil in step 7.

7. Seal the OpenArray™ 384-well Sample Plate with an aluminum foil seal, remove the foil flap, then mark the edges of the filled 4 × 12 area with a pen.

8. Vortex the plate for 20–30 seconds, then centrifuge the plate at 1,200 × *g* for 1 minute.

9. Score the foil along the lines that were marked before centrifuging.

   Do not remove the foil from the scored area at this time.

If you make a sample layout error before the AccuFill™ procedure – Repeat "Generate OpenArray™ Plate layouts in the OpenArray™ Sample Tracker Software" on page 77 with a corrected 96-well sample CSV file.

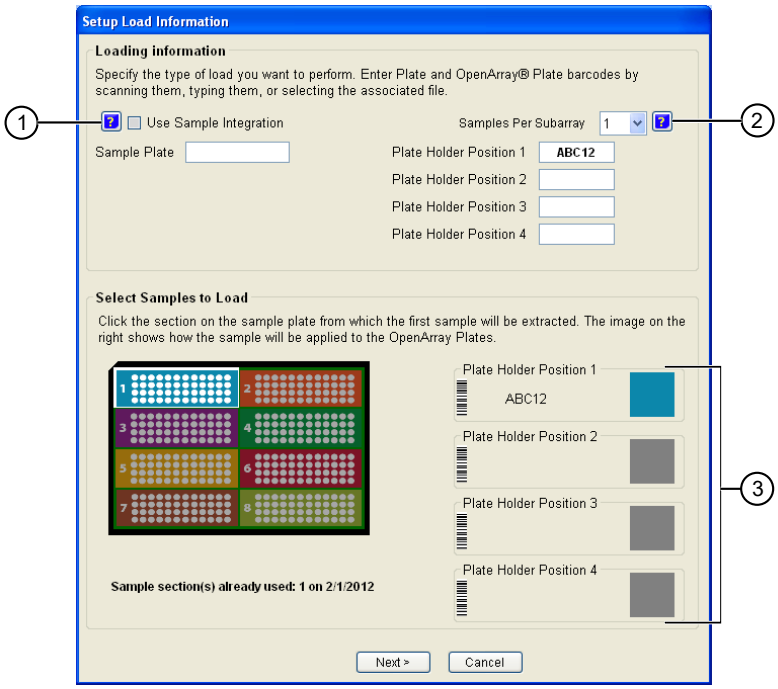
Set up the OpenArray™ AccuFill™ Instrument and the OpenArray™ AccuFill™ Software

IMPORTANT! Do not use OpenArray™ AccuFill™ System Tips that exceed the expiration date (shown on the outer box that contains the tip trays).

1. In the OpenArray™ AccuFill™ Software, click **Setup and Load**.
   The **Setup Load Information** window appears.

2. In the **Setup Load Information** window, select the number of samples per subarray, then enter the OpenArray™ Plate barcodes in the appropriate plate holder positions.

   Note: Ensure that the **Use Sample Integration** checkbox is deselected.



① **Use Sample Integration**
② **Samples Per Subarray**
③ **Plate Holder Position** corresponding to the position of the OpenArray™ Plate on the deck of the instrument.

3. In the **Select Samples to Load** pane (bottom section of the window), click the corresponding 4 × 12 area of the 384-well sample plate image, then click **Next**.
   The **Setup Deck** window is displayed.

4. In the OpenArray™ AccuFill™ Instrument, ensure that:
   - Tip boxes and tips are loaded as shown in the **Setup Deck** window.
   - The lids are removed from the tip boxes.
   - The waste bin in the instrument is emptied.

5. In the **Setup Deck** window, confirm that the deck is ready:
   - Select **The tips are configured as shown above**.
   - Select **The Waste Bin is empty**.

Transfer reactions to the OpenArray™ Plate using the OpenArray™ AccuFill™ Instrument

IMPORTANT! Ensure that the OpenArray™ Plate is thawed and that the entire plate is at room temperature.

1. Prepare the items needed to seal the loaded OpenArray™ Plate (next section).

   Note: The OpenArray™ Plate must be sealed promptly after being loaded with the reactions, as described here.

   - Ensure that the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0 is ready.
   - Gather and remove from their packaging the following: an OpenArray™ Lid, plug, syringe with OpenArray™ Immersion Fluid, and syringe tip.
   - Attach the syringe tip to the syringe, carefully push some of the fluid through the tip to remove air bubbles, then lay the syringe aside.

2. Load the OpenArray™ Plate and the OpenArray™ 384-well Sample Plate into the OpenArray™ AccuFill™ Instrument.
   - **OpenArray™ Plate**—Remove the plate from its sleeve, then place the plate in the appropriate plate holder position in the instrument.

     Ensure that the barcode on the OpenArray™ Plate is facing left and the serial number is facing right.
   - **OpenArray™ 384-well Sample Plate**—Place the 384-well sample plate onto the deck of the instrument, then use forceps to peel the foil from the filled area of the plate.

3. Close the door of the instrument.

4. In the OpenArray™ AccuFill™ Software **Setup Deck** window, select the following confirmations:
   - **The OpenArray Plate is in the Plate Holder**
   - **Remove foil from the highlighted section of the Sample Plate**

5. Click **Load**.

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.

   See “Seal and run the OpenArray™ Plates” on page 91.

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

Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v2.0

For required materials, see “Required materials for the OpenArray™ Plate workflow” on page 74.

For instructions for OpenArray™ AccuFill™ Software v1.2, see “Prepare the OpenArray™ Plates with OpenArray™ AccuFill™ Software v1.2” on page 76.

This chapter describes the full run workflow. For other workflow options, see the following documentation.

This chapter contains brief procedures. For detailed procedures, see the following documentation.

Document	Pub. No.
QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Experiments User Guide	4470935
QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide	MAN0025669
OpenArray™ AccuFill™ Software v2.0 Quick Run Workflow Without Sample Information Quick Reference	MAN0025835
OpenArray™ AccuFill™ Software v2.0 Full Run Workflow Quick Reference	MAN0025836

Download SPF files

To prepare an array-specific template EDT file, you only need to download an SPF file once. If there is a change to the plate layout, you will need to download a new SPF file, then create a new template.

The computer with the OpenArray™ AccuFill™ Software v2.0 must be connected to the internet.

1. In the **TPF/SPF** screen of the OpenArray™ AccuFill™ Software v2.0, select the **Download** radio button.
2. Select the product.
   - **TaqMan OpenArray Custom**
   - **TaqMan OpenArray Inventoried**

3. Enter the following information.

Product	Information
TaqMan OpenArray Custom	a. Enter the ***Lot number*** or ***Batch number***. b. Enter one ***Serial number*** from the lot. **Note:** Only one serial number is required. The serial number is used to confirm the lot number or batch number. All of the files in the lot or batch are downloaded.
TaqMan OpenArray Inventoried	Enter the list of ***Serial numbers*** or ***Barcodes***. Separate more than one serial number or barcode with a comma or a line break. **Note:** The serial number or barcode entered corresponds to the file that is downloaded. Enter a serial number or barcode for each file to download.

Note: The fields that are displayed depend on the product selected in step 2.

4. Click **Download**.

The location of the files is displayed at the top of the screen. The location of the downloaded files is set in the **Preferences** menu, in the **OpenArray plate file folder** field. See *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669) for more information about setting the preferences.

The files are in a compressed ZIP folder.

Click **Open folder** to access the files or click ✕ **(Close)** to close the message.

Extract the files from the compressed ZIP folder.

Create an array-specific template (EDT) file

1. In the **Home** screen of the QuantStudio™ 12K Flex Software, in the **Experiment** pane, click **Create From Template**.

   An **Open** window will appear.

2. In the **Look in** field, select **OpenArray**, click **Genotyping** (file name **Genotyping.edt)**, then click **Open**.

   A new experiment is created using the setup information from the template.

3. From the open experiment, click the **Targets** tab on the left pane, then click **Import** ▸ **Import Plate Setup**.

4. Click **Browse** to select the SPF plate file for the desired panel, then click **Select**.

5. Click **Start Import** ▸ **Yes** to confirm the import.

6. Click **Save As Template** to save the array-specific EDT file.

7. Click **Save**.

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

IMPORTANT! The 4 × 12 areas of the OpenArray™ 384-well Sample Plate being filled must match the areas designated in the OpenArray™ AccuFill™ Software for that set of samples.

1. Remove an OpenArray™ Plate from the freezer and set it aside. 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 OpenArray™ 384-well Sample Plate created in this section.

2. Gently swirl the contents of the TaqMan™ OpenArray™ Genotyping Master Mix to thoroughly mix. Do not invert the bottle.

3. 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.

Component	OpenArray™ Plate Format		
	16	32	64+
	Volume per well		
TaqMan™ OpenArray™ Genotyping Master Mix	1.5 µL	2.0 µL	2.5 µL
One of the following: • 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**

4. Thoroughly mix each PCR reaction by pipetting up and down or by using the "mix" function on a multi-channel pipette. Alternatively, vortex the OpenArray™ 384-well Sample Plate for 10–15 seconds after sealing with aluminum foil in step 5.

5. Seal the OpenArray™ 384-well Sample Plate with an aluminum foil seal, remove the foil flap, then mark the edges of the filled 4 × 12 area with a pen.

6. Vortex the plate for 20–30 seconds, then centrifuge the plate at 1,200 × *g* for 1 minute.

7. Score the foil along the lines that were marked before centrifuging.

   Do not remove the foil from the scored area at this time.

If you make a sample layout error, it is possible to correct this in the OpenArray™ AccuFill™ Software using the plate rotation feature. For more information, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

Map and export plate files

Configure the experiment design to map the plates

A sample plate file (CSV file) is *not* required.

Navigate to the **Map Plates** screen.

1. In the **Configure design** pane, in the **Experiment type** section, select **Genotyping**
2. In the **Plate format** section, select a value.
3. In the **Sample input type** section, select the type of plate.
   - **96-well**
   - **384-well**
4. In the **Pipettor** section, select a type of pipette.
   - **Fixed**
   - **Adjustable**
5. Click **Next**.

The **Map plates** pane is displayed.

If **96-well** was selected in step 3, proceed to “Map a 384-well plate from 96-well plates” on page 85.

If **384-well** was selected in step 3, proceed to “Set up a 384-well sample plate” on page 86.

Map a 384-well plate from 96-well plates

1. In the **Map Plates** pane, add the sample name in the 96-well plate.
   Click and drag to copy and paste sample names across a row or down a column.

   Note: The sample names are copied. They are not automatically filled. For example, if **Sample 1** is the first sample name, all of the wells that are filled by the click and drag feature are **Sample 1**. They are not named sequentially, for example, **Sample 1**, **Sample 2**, **Sample 3**.

2. *(For Genotyping 256 plate formats)* In the 96-well plate, select a sample run.
   - **Sample Run 1**
   - **Sample Run 2**

   If **Sample Run 1** is selected, the top half of the 96-well plate is defined with the samples. If **Sample Run 2** is selected, the bottom half of the 96-well plate is defined with the samples.
   This feature allows for two runs with a 96-well plate, if only 48 wells of the physical plate are used.

3. *(Optional)* In the 384-well plate, click **Swap**.
   This moves the samples from the top half of the 384-well plate to the bottom half of the 384-well plate.

Note: The **Swap** button is displayed only if certain experiment types and plate formats are selected. It is displayed when half of the 384-well plate is used.

4. *(Optional)* Click **Import** to import a sample file (CSV format).

   A sample file can be imported from the **Map plates** pane if it was not imported from the **Configure design** pane.

   Note: If a sample plate file is imported from the **Map plates** pane, the information will overwrite any information from files that were imported from the **Configure design** pane.

Proceed to "Export plate files" on page 87.

Set up a 384-well sample plate

If the sample plate file was imported, the sample names are displayed. The sample plate layout defined in the sample plate file can be edited.

If the sample plate file was not imported, the samples must be added manually.

The plates are color-coded for the position on the OpenArray™ Plate based on how the experiment is configured.

1. In the **Map Plates** pane, add the sample name to the 96-well plate.

   Click and drag to copy and paste sample names across a row or down a column.

   Note: The sample names are copied. They are not automatically filled. For example, if **Sample 1** is the first sample name, all of the wells that are filled by the click and drag feature are **Sample 1**. They are not named sequentially, for example, **Sample 1**, **Sample 2**, **Sample 3**.

2. *(Optional)* Click **Import** to import a sample plate file (CSV format).

   A sample plate file can be imported from the **Map plates** pane if it was not imported from the **Configure design** pane.

   Note: If a sample plate file is imported from the **Map plates** pane, the information will overwrite any information from files that were imported from the **Configure design** pane.

3. *(Optional)* Click **Rotate data**.

   The 384-well plate can be placed inside the OpenArray™ AccuFill™ Instrument in only one orientation. If the samples were added to the physical 384-well plate in the incorrect orientation, the physical 384-well plate cannot be rotated when it is placed in the instrument for the sample plate file to match. The option to rotate the samples in the sample plate file allows the 384-well plate to be used in the correct orientation for the instrument with a matching sample plate file.

   The **Rotate data** feature is available only in the **Map Plates** screen if a 384-well sample plate file was imported. It is not accessible in the full run workflow.

Proceed to "Export plate files" on page 87.

Export plate files

1. In the **Map plates** pane, click **Export**.
   The **Select Plates to Export** dialog box is displayed.
2. Select **CSV file** as the file type to export.
3. Select the **OpenArray Plate (for QuantStudio)** as the plate file to be exported
4. Enter a prefix for the exported file name in the **File Name Prefix** field.
5. Click **OK**.

Set up and start the run with the quick run workflow

Before you begin—quick run workflow

- Prepare samples (DNA and PCR reaction mix) in a 384-well plate, according to the user guide or the application guide for your assay. Distribute the samples according to the layout determined in the **Map Plates** workflow.
- Place the sample plate in the sample plate holder on the instrument deck, with the notch to the left.
  Do not stack sample plates.
- Load the tip boxes, then remove the tip box covers.
  Do not stack the tip boxes.
- Place the OpenArray™ Plate in the plate holders.
- Clear the instrument deck, empty and replace the waste bin, and close the instrument door.
- Prepare the QuantStudio™ 12K Flex OpenArray™ Accessories Kit materials that are required to seal the OpenArray™ Plate.

  IMPORTANT! OpenArray™ Plates must be sealed immediately after loading. For more information, see the user guide or application guide for your assay.

Configure the run without sample plate information

A sample plate file (CSV file) is *not* required by the OpenArray™ AccuFill™ Software v2.0.

Navigate to the **Quick Run** screen. Ensure that the **Load without sample plate information** radio button is selected.

1. *(Optional)* In the **Quick Run** screen, in the **Sample plate - optional** field, enter information to identify the sample plate.
   - Enter the information about the sample plate.
   - Use a barcode scanner to scan the sample plate or manually enter the barcode text string.

2. Select the number of samples per subarray.
   - One
   - Two
   - Three
3. In the **OpenArray Plate name and position** field, enter information to identify the OpenArray™ Plate.

   We recommend using the serial number of the OpenArray™ Plate as the identifying information.

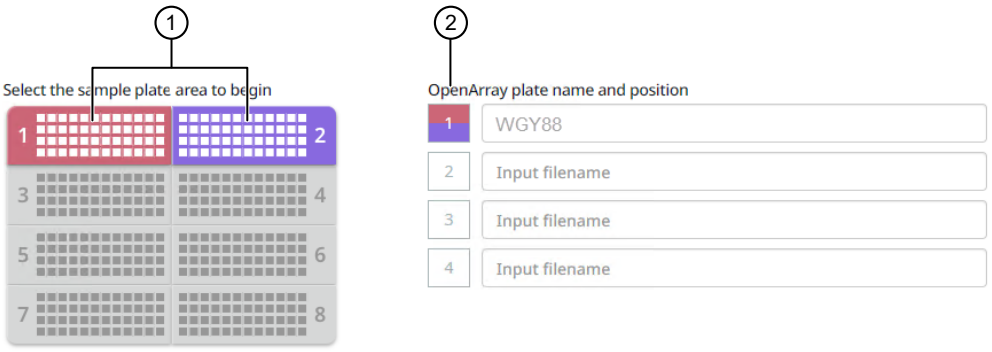
   The name and position are recorded in the loading history log.
4. Click a section of the sample plate to change the corresponding OpenArray™ Plate.

   Note: The first section of the sample plate is selected. Subsequent sections are selected automatically.

   During the run, the instrument fills the OpenArray™ Plate with the samples from the corresponding section of the sample plate.

   The OpenArray™ Plate position displays the color that corresponds to the section of the sample plate.

   If there is more than one sample in a subarray, the position box displays all the colors associated with the corresponding sample.



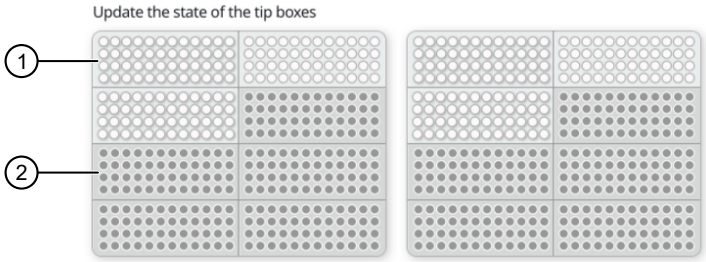
① Sample plate section
② Corresponding OpenArray™ Plate

Verify the run setup and start the run

1. Click each tip box so that the status on the **Verify the run setup and start the run** section matches the physical tip box in the instrument.

   We recommend starting the run with full tip boxes.

   The instrument does not start the run if there are not enough tips on the deck.



① Section of the tip box is full
② Section of the tip box is empty

2. Select the first section of the sample plate to fill the OpenArray™ Plate.

   Select the first section of the sample plate if multiple OpenArray™ Plates are filled during a run. The software selects the total number of sections that correspond with the total number of OpenArray™ Plates.

3. Remove the foil from the appropriate sections of the sample plate, then click the checkbox to confirm.

   Remove the foil only from the sections of the sample plate that are used to load a single OpenArray™ Plate.

   The number of sections that the foil is removed from depends on how the run was set up. For example, if the run was set up without sample information and for two samples per subarray, the foil is removed from two sections at a time.

   Note: Do not remove the foil from all the sections of the sample plate at once.

4. Close the instrument door.

5. Click **Start Run**.

   The run does not begin under any of the following conditions:

   - The waste bin is not in position
   - The sample plate is not in position
   - The OpenArray™ Plates are not in position
   - There are more OpenArray™ Plates on the instrument deck than are defined in the experiment setup

The **Deck** screen is displayed.

IMPORTANT! Each OpenArray™ Plate must be prepared for PCR immediately after it is filled.

Remove the OpenArray™ Plate from the instrument

1. Open the instrument door and remove the OpenArray™ Plate that is indicated by the blue box in the dialog box.

   IMPORTANT! Remove the OpenArray™ Plate within 30 seconds, to avoid evaporation within the plate.

One of the following dialog boxes is displayed:

- The **Remove plate and foil** dialog box.
- The **Remove plate** dialog box (after the last OpenArray™ Plate is filled).

2. Seal the case and fill the OpenArray™ Plate with immersion fluid.
   See "Seal the OpenArray™ Plate" on page 91.

3. *(For **Remove plate and foil** dialog box only)* Remove the foil seal from the next section of the sample plate, then select the checkbox to confirm that the foil is removed from the section of the plate that is highlighted.

   Note: Remove the foil only from the next section of the sample plate. Do not remove the foil from all sections of the sample plate.

4. Close the instrument door.

5. Click **Done**.
   The run does not proceed under any of the following conditions:
   - The waste bin is not in position
   - The sample plate is not in position
   - The OpenArray™ Plates are not in position
   - There are more OpenArray™ Plates on the instrument deck than are defined in the experiment setup

   The instrument loads the next OpenArray™ Plate.

6. Repeat step 1to step 5 for each OpenArray™ Plate to be loaded.
   See "Seal and run the OpenArray™ Plates" on page 91.

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

After all of the OpenArray™ Plates have been loaded, the **Deck** screen displays **Run completed successfully. Empty the waste bin before performing another run.**

Seal and run the OpenArray™ Plates

Seal the OpenArray™ Plate

IMPORTANT! Throughout this procedure, handle the OpenArray™ Plate and the OpenArray™ Case only by the edges.

Note: The OpenArray™ Case consists of the sealed OpenArray™ Plate and the OpenArray™ Lid.

1. Place the newly loaded OpenArray™ Plate in the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0.

   Ensure that the barcode is facing left and the serial number is facing right.

2. From the OpenArray™ Lid, remove the clear protective film from the *inside* of the lid ① and the red adhesive-protective strip ② from around the edge of the lid.

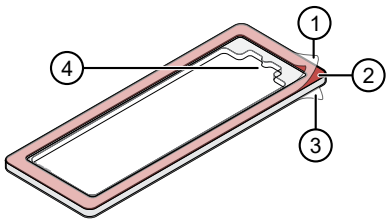
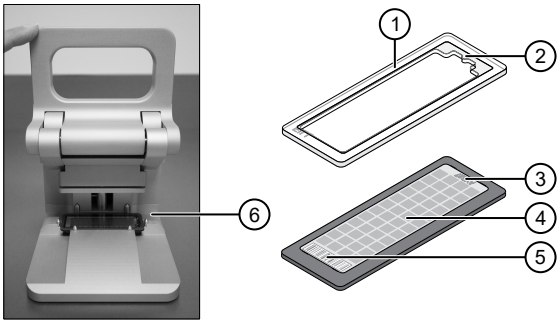


Figure 4 OpenArray™ Lid

① Protective film on inside of the lid (remove before *sealing*)
② Red adhesive-protective strip (remove before *sealing*)
③ Protective film on the outside of the lid (remove before *running*)
④ Notched end (align with serial number on plate)

3. Place the lid in the Plate Press using the alignment pins of the Plate Press for orientation.

IMPORTANT! The notched end of the case lid must be oriented towards the furthest back right-side of the Plate Press.



① OpenArray™ case lid
② Notched end of lid
③ Serial number of plate
④ OpenArray™ Plate
⑤ Barcode of plate
⑥ Alignment pins

4. Seat the lid on the OpenArray™ Plate with the lid adhesive against the plate.

5. Engage the press mechanism until the green flashing light changes to a steady green light (after 20 seconds).

   The status light turns solid green, indicating that the case is sealed.

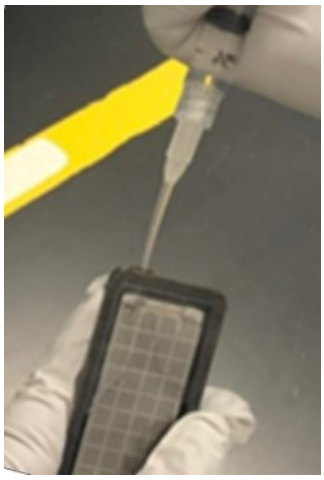
Note: Do not apply additional pressure onto the Plate Press during its actuation.

6. Disengage the press and carefully remove the OpenArray™ Case.

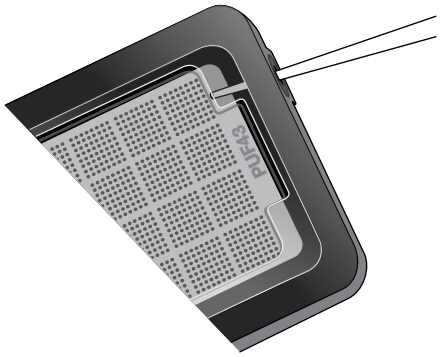
7. Prepare the immersion fluid. Remove the cap, insert the accompanying syringe tip, and prime the syringe by ejecting a small amount of immersion fluid onto a paper towel to ensure no air gap remains in the newly attached pipette tip.

IMPORTANT! If the syringe is not primed, the direct burst of air and fluid can negatively affect the assay(s) at the end of the array.

8. While holding the case upright by its edges at a 15–30 degree angle so that the port is at the highest point of the array, insert the prepared syringe tip into the port in the case.



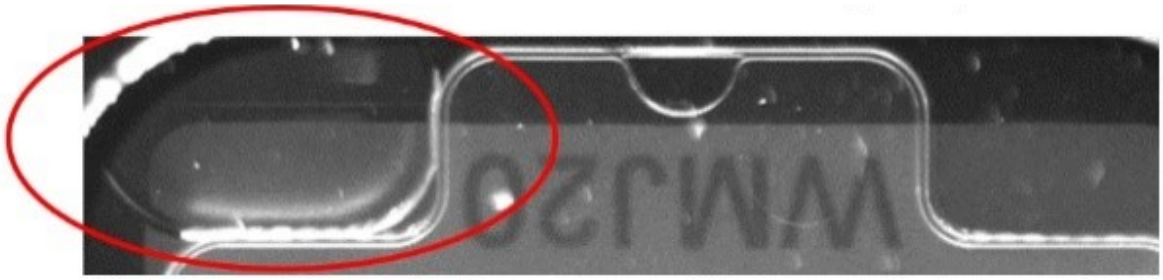
The syringe tip must be in front of the array when filling the case with immersion fluid.



9. Slowly inject the OpenArray™ Immersion Fluid until the case is filled, which should take about 10 seconds to fill. Minimize the creation of additional air bubbles when you dispense the fluid. Leave a small air bubble as shown below.

IMPORTANT! If injected too quickly, the fluid can flush out the samples that are suspended in the through-holes.

Overfilling the array and/or not leaving a small bubble may cause a leak during the PCR run.



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

    Note: Ensure that you are screwing the plug in at the same angle the case base is at. If it is off, it can cause the plug to break off prematurely.

    IMPORTANT! To avoid leaking of immersion fluid, hold the case *vertically* and rotate the plug slowly to avoid cross-threading.

    If the plug handle breaks off prematurely, use a Phillips #0 screwdriver to complete this step. Do not overtighten. If plastic or adhesive remains attached to the screw due to premature breakout of the plug handle, remove it with forceps prior to loading it into the instrument.

11. If needed, clean the case with the lint-free cloth included with the OpenArray™ Plate or a laboratory wipe that has been thoroughly sprayed with ethanol, then dry the case with a clean laboratory wipe.

Integrate sample names before or after the run

Note: You can choose to integrate samples names *before* or *after* the run. If you choose to integrate samples names *after* the run, proceed immediately to "Run an array-specific template EDT or EDS file" on page 95.

1. At the top of the screen, click **Tools** ▸ **Preferences**.
   The **Preferences** window is displayed.
2. Click the **OpenArray** tab.
3. Next to the **Setup Folder** field, click **Browse**.
   a. Open your experiments folder.
   b. Create and name a new folder within the experiments folder (e.g., ***Templates for OA***).
4. In the **Home** screen of the QuantStudio™ 12K Flex Software, in the **Experiment** pane, click **Open**.
   a. Navigate to the folder you made in substep 3b.
   b. Select the desired EDT file, then click **Open**.
5. In the **Setup** pane, click the **Samples** tab, then click **Import**.
   a. Select the OpenArray™ Plate sample information CSV generated from the OpenArray™ Sample Tracker Software or Map Plates.
   b. Click **Select File**.
6. In the **Setup** pane, click the **Experimental Properties**.
   a. In the **Experiment Name** field, enter in the experiment name.
   b. In the **Barcode** field, enter in the serial number of the plate you are running.

7. At the top of the screen, click **Save** ▸ **Save As** to save the file in EDS format.
8. Click **Save**.

Run an array-specific template EDT or EDS file

1. In the **Home** screen of the QuantStudio™ 12K Flex Software, in the **Run** pane, click **OpenArray**.
2. In the **Setup OpenArray Run** pane, click **Get Plate IDs**.
   The plate ID information will populate. This may take several minutes.
3. Click **Browse** next to the **Setup File** field.
4. Under the **Files of type** dropdown menu, change the file type from SPF to EDT.

   Note: Change the file type from SPF to EDS if you completed "Integrate sample names before or after the run" on page 94.
5. Select the desired EDT or EDS file, then click **Select**.
6. *(Optional)* Click **Browse** to change the **Experiment File Location**.
7. *(Optional)* Change the software-determined **Experiment File Name**.
8. Click **Start Run**.
9. Transfer the EDS file from the instrument to an accessible location for analysis.
10. Check the QC images for loading issues or leaks.

Note: If you did not integrate samples names *before* the run, proceed to "Integrate sample names before or after the run" on page 94.

Check the quality–control images

Check the quality–control (QC) images before analysis. Images can be viewed using ImageJ, an open–source software available from the NIH at **imagej.nih.gov/ig**.

1. In the QuantStudio™ 12K Flex Software **Export** screen, click **Browse**, then create a uniquely–named folder for the QC images export.

   IMPORTANT! Create a new folder for images each time. Exporting a second run to the same folder overwrites the images.
2. Click **Export QC Images** at the bottom of the screen.
3. View the following ROX™ image to check for loading quality issues:
   - POST-READ_CHANNEL_4.tiff

4. Check the following spotfinding images for leaks or other displaced sample issues.
   - s02_c001_t03_p0001_m1_x2_e1_cp#_spotfind.tiff
   - s02_c040_t03_p0001_m1_x2_e1_cp#_spotfind.tiff

   Note: The “cp#” in the image file name refers to array positions 1 through 4 within the instrument.

5. If a problem is found, view the following pre-run spotfinding image to determine whether the issue existed before cycling:
   - s00_c001_t01_p0001_m2_x3_e1_cp#_spotfind.tiff

6. View the following FAM™ images to check for fluorescent abnormalities and to confirm any problem seen in the spotfinding images:
   - STAGE2_CYCLE1_CHANNEL_1.tiff
   - STAGE2_CYCLE40_CHANNEL_1.tiff

7. Note any abnormalities found, as well as all other potentially relevant information related to the setup of the run.

6 Analyze OpenArray™ PGx SNP genotyping experiments

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

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

Before you begin analysis (each time)

Transfer files from QuantStudio™ 12K Flex Real–Time PCR System software with a network connection

1. In the Home screen of the QuantStudio™ 12K Flex Real–Time PCR System Software, click **Instrument Console**.
2. In the Instrument Console, select the desired QuantStudio™ 12K Flex Real–Time PCR System from the list of instruments on the network, then click **Add to My Instruments**.
3. After the QuantStudio™ 12K Flex Real–Time PCR System 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 Real–Time PCR System:
   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, see 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 Real–Time PCR System 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 Real–Time PCR System software	• Desktop software • View real-time trace data to aid genotype calling
Thermo Fisher™ Connect Genotyping Application	• Cloud software • Create studies • Overlay data from multiple plates • View real-time trace data to aid genotype calling
TaqMan™ Genotyper Software	• Desktop software • Create studies • Overlay data from multiple plates

Analyze data in QuantStudio™ 12K Flex Real–Time PCR System or Connect software

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

To use the QuantStudio™ 12K Flex Real–Time PCR System 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 Connect 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.

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 SNP Assays.

For best results, generate a new reference panel for each lot of SNP Assays.

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 100.
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	a. Select **Analysis** ▸ **Quality Control**. b. 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. c. (*Optional*)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	a. Select **Analysis** ▸ **Quality Control**. b. Review call rates and QC data in the **Experiments** tab.
Review assay call rates and QC data	a. Select **Analysis** ▸ **Quality Control**. b. 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'.

Manually determining genotype:

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 12 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

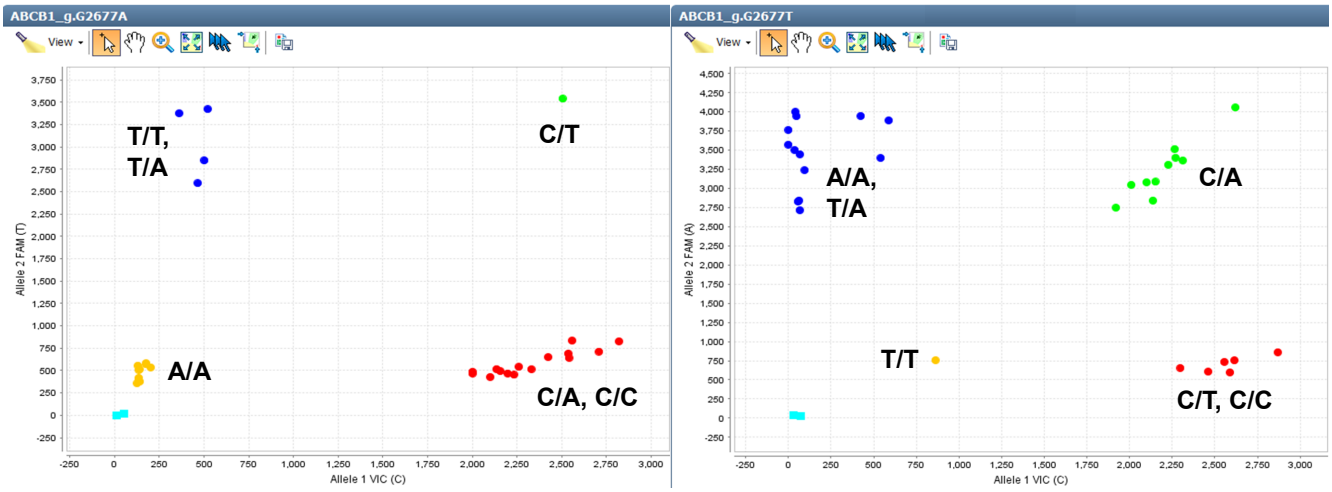


Figure 5 Triallelic SNP rs2032582 assay results Left: ABCB1 c.3095G>A assay (C_11711720D_40) to the plus strand C wild type and T mutant allele. Right: ABCB1 c.3095G>T assay (C_11711720C_30) to the C wild type and A mutant allele.

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

Haplotype/ Haplotype	Diplotypes[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

Table 13 Translation table for the CYP2C19*2,*10 adjacent SNP assays *(continued)*

Haplotype/ Haplotype	Diplotypes[1]	*2 assay 681 G/A - 680C	*10 assay 681G - 680 C/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, diplotypes containing A-T haplotypes are not included.

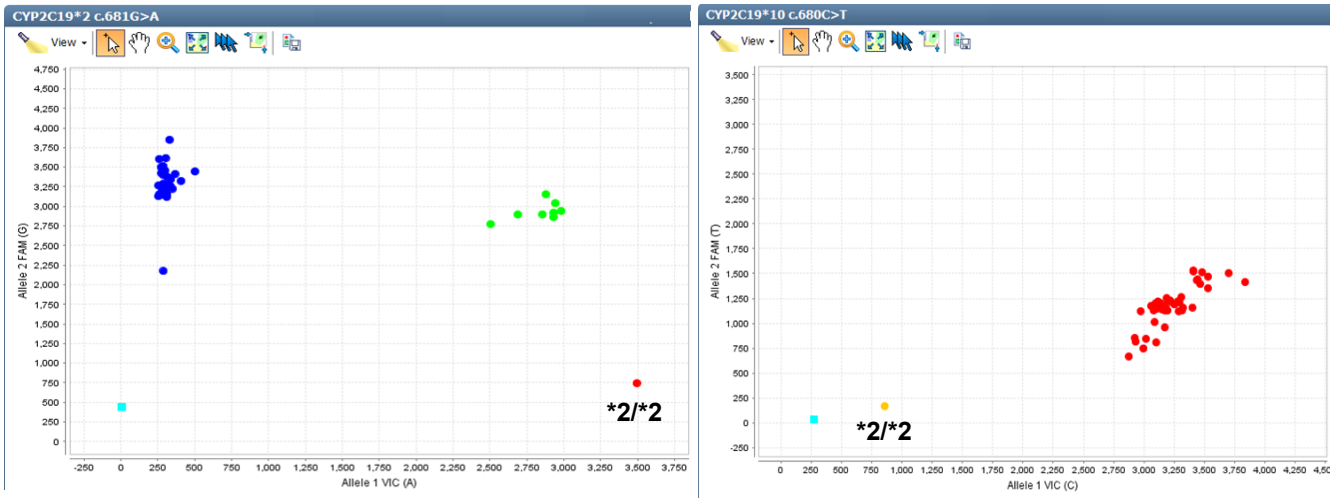


Figure 6 CYP2C19*2 and *10 assay OpenArray™ results Left: rs4244285 CYP2C19*2 681G>A assay (C__ 25986767_70) to the G wild type and A mutant allele. Right: rs6413438 CYP2C19*10 c.680C>T assay (C__30634128_10) to the C wild type and T mutant allele. Samples: Coriell gDNAs; NA17263 has a *2/*2 diplotype that cannot be detected by the *10 assay.

Table 14 Translation table for the CYP2C9*3,*4 adjacent SNP assays

Haplotype/ Haplotype	Diplotypes[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, diplotypes containing A-A haplotypes are not included.

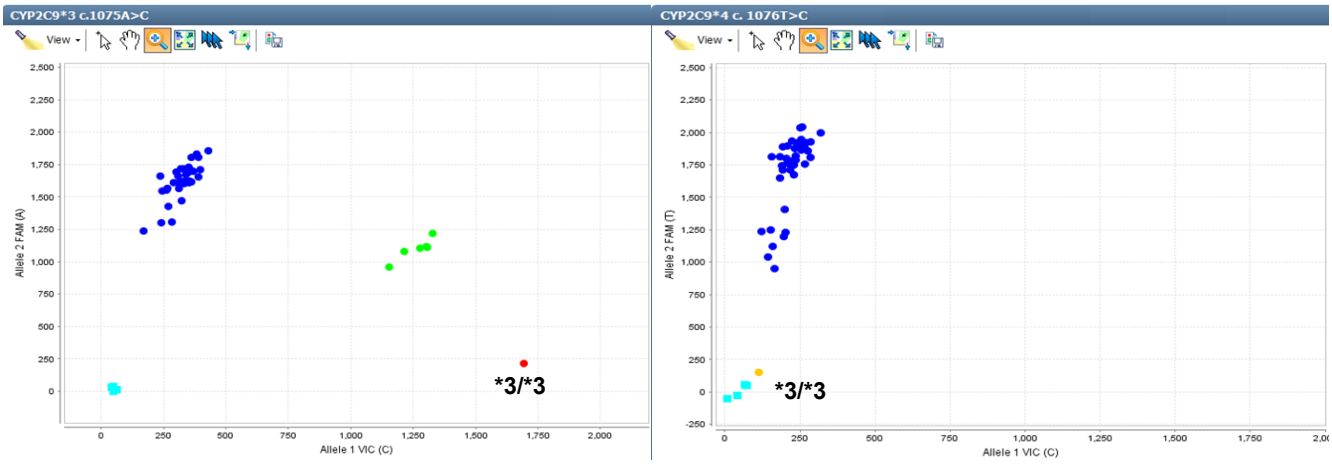


Figure 7 CYP2C9*3 and *4 assay OpenArray™ results Left: rs1057910 CYP2C9*3 c.1075A>C assay (C__27104892_10) to the A wild type and C mutant allele. Right: rs56165452 CYP2C9*4 c. 1076T>C assay (C__30634131_20) to the T wild type and C mutant allele. Samples: Coriell gDNAs; NA17247 has a *3/*3 diplotype 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.
TaqMan™ Genotyper Software Getting Started Guide	4448637
TaqMan™ OpenArray™ Genotyping Troubleshooting Guide	MAN0011115

7 Prepare, run, and analyze PGx copy number experiments

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

Title	Pub. No.
TaqMan™ Copy Number Assays User Guide	4397425
CYP2D6 Allele-specific Copy Number Analysis Quick Reference	MAN0011114
CopyCaller™ Software v2.0 User Guide	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 User Guide* (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 CT 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 `SampleFile_384-Well Plate_192-Format.xlsx` 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/µL in nuclease-free water or 1X TE buffer in a total of 10 µL.

  Use the `SampleFile_384-Well Plate_192-Format.xlsx` to calculate the dilutions based on the concentration determined from the RNase P assay.

- Generate the CNV Sample .txt file from the `SampleFile_384-Well Plate_192-Format.xlsx`.

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 µL of 60X assay with 20 µL 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-µL reaction[1]
TaqPath™ ProAmp™ Master Mix[2]	5.0 µL
TaqMan™ Copy Number Assay (20X)	0.5 µL
TaqMan™ Copy Number Reference Assay (20X)	0.5 µL
Nuclease-free water	2.0 µL
Total volume	**8.0 µL**

[1] Use the `SampleFile_384-Well Plate_192-Format.xlsx` 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 µL of the reaction mix to the sample and control wells of a 384-well reaction plate.

4. Vortex the normalized gDNA samples (5 ng/µL), add 2 µL 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.

Perform real-time PCR

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™ dye	MGB-NFQ
TaqMan™ Copy Number Reference Assay	VIC™ dye	TAMRA™ dye

3. In the **Assign** screen:
   - Import the CNV Sample TXT file generated from the `SampleFile_384-Well Plate_192-Format.xlsx`, 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 µL.
   - Confirm the following thermal cycling parameters.

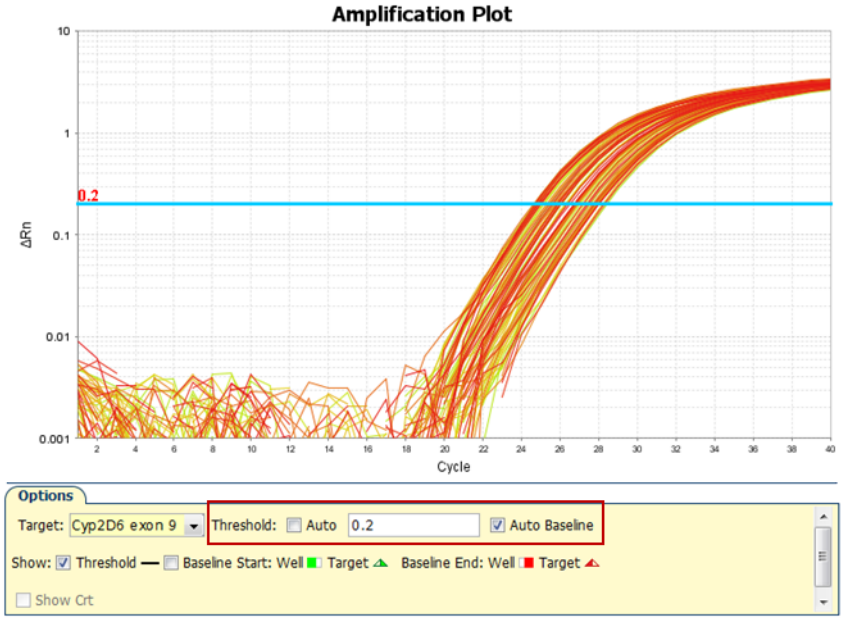
Stage	Step	Temperature	Time
Hold	Step 1	95°C	10 minutes
PCR (40 cycles)	Step 1	95°C	15 seconds
	Step 2	60°C	60 seconds

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 113 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.	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.	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).
Calibrator	Copy number results are based on the copy number designated for one calibrator sample. Include a calibrator sample on each plate. **IMPORTANT!** The calibrator should be of the same sample type as the test samples.	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.

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

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.

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.
- The samples cluster with passing samples of the same copy number group in the ΔC_t plot.

A 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.

Troubleshoot with cycling and imaging run images (QC 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™ dye images.

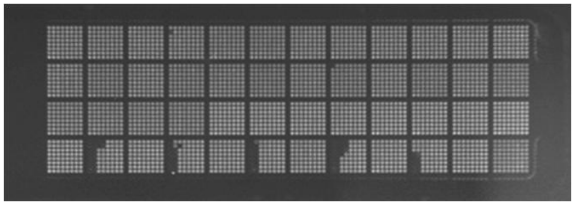
(continued)

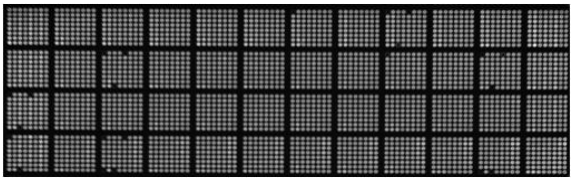
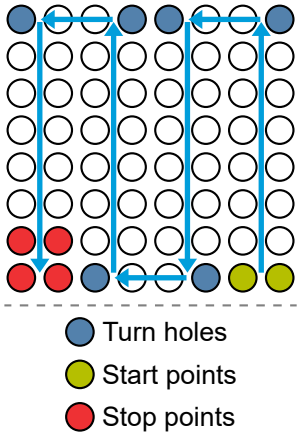
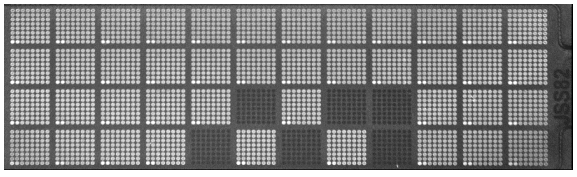
To...	View image...	Image description
Check for existing contamination on the case and/or heated cover	s00_c001_t01_p0001_m2_x3_e1_cp#_spotfind.tiff[1]	Pre-run reflected light spotfinding image (used by the software to determine the location of the holes).
Identify potential leaks or other contamination	s03_c001_t03_p0001_m2_x3_e1_cp#_spotfind.tiff[1]	Mid-run reflected light spotfinding image.
	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 Real-Time PCR Instrument.

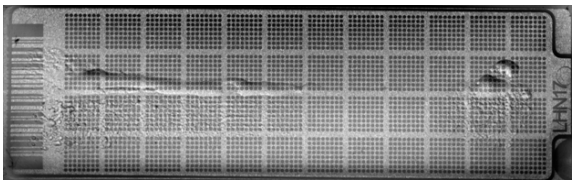
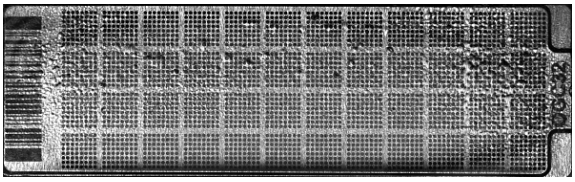
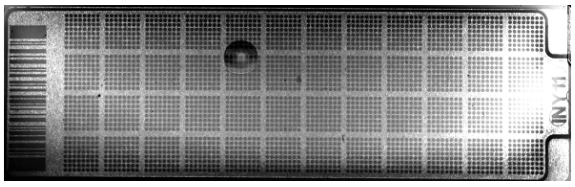
3. *(Optional)* Adjust the images for brightness and/or contrast to make image features visible.
   a. Open the image in ImageJ.
   b. Select **Image ▸ Adjust Brightness/Contrast** (or press **Ctrl+Shift+C**).
   c. Click **Auto** or adjust the sliders until the features of interest in the image are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

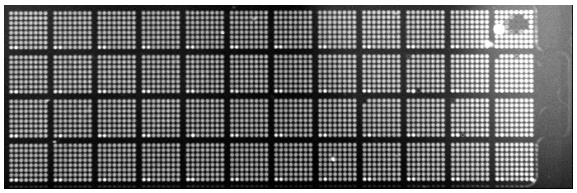
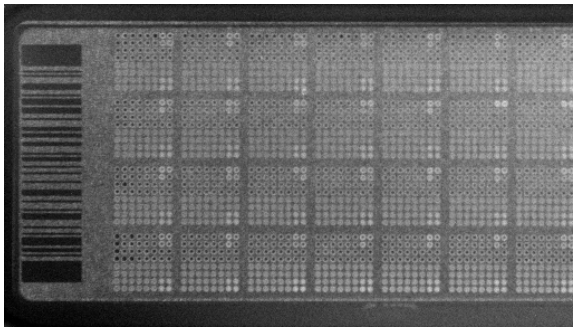
OpenArray™ AccuFill™ Instrument plate loading errors

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 plate at 1,200 × *g* for 60 seconds.

Observation	Possible cause	Recommended action
Turn-holes are repeatedly missed 	The OpenArray™ AccuFill™ Instrument is aligned too far to the left or to the right. Systematic loading problems can occur with the OpenArray™ 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 instrument changes direction during sample loading. 	Contact your local field service engineer.
Entire subarrays are missing 	The sample/master mix was not added to particular wells in the 384-well Sample Plate.	Visually inspect the plate to ensure that the wells have sample/master mix.
	Stuck tip mandrel on the OpenArray™ AccuFill™ Instrument may need cleaning.	Contact your local field service engineer.
	Pipette tip was not loaded on mandrel.	Contact your local field service engineer for frequent occurrences (infrequent occurrences can be due to a poorly molded tip).

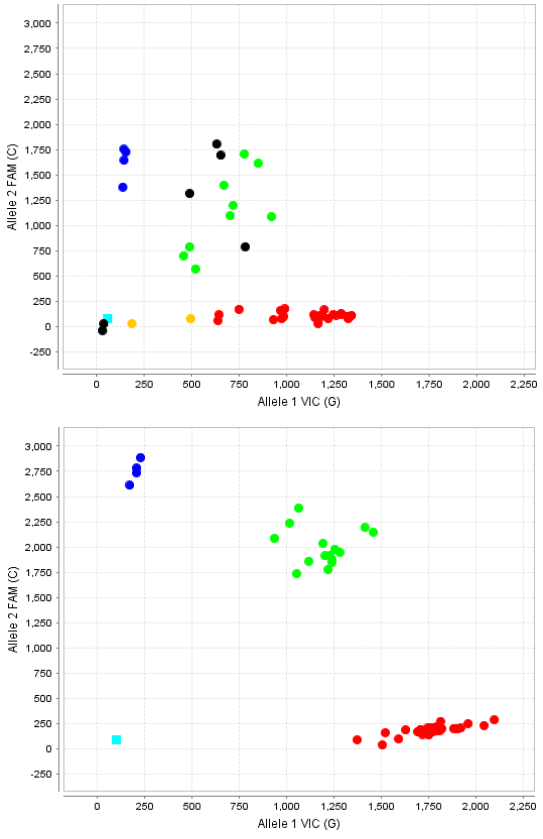
OpenArray™ Plate assembly and handling errors

Observation	Possible cause	Recommended action
Case leaks and bubbles inside the case    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 data. 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. 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 (QC images)” on page 115.	Plate press was not engaged for at least 20 seconds.	Fully engage the plate press for at least 20 seconds.
	Damaged lid adhesive.	Remove the liner and visually inspect the lid adhesives for defects. Ensure that adhesive is not damaged or warped.
	Damaged fill port screw gasket.	Visually inspect the screw to ensure that the orange gasket is present and not damaged.
	Damaged fill port screw assembly. Breaks off too easily.	The screw may be mis-threaded: unscrew it and use a new screw assembly.
	Oily lid or case from immersion fluid overflow.	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).
	Immersion fluid was exposed to air for too long.	Do not remove the immersion fluid syringe cap or draw air bubbles into the syringe until you are ready to load.
	Too large of a bubble inside the OpenArray™ Case after sealing.	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.
	Damaged plate press, leading to uneven pressure.	Contact your field service engineer if you suspect that your plate press may be damaged.

Observation	Possible cause	Recommended action
Sample blow-out during the addition of immersion fluid 	The reactions in A12 were compromised during the addition of immersion fluid. Injecting the immersion fluid too quickly can 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.	Dispense a small amount of immersion fluid onto a paper towel before use to ensure smooth operation of the syringe.
Evaporation of reaction mixture in through-holes 	Too much time elapsed before the 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 though-holes.	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.

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.	We recommend using 50 ng/µL as the 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 *TaqMan™ OpenArray™ Genotyping Sample Preamplification User Bulletin* (Pub. No. MAN0011116). The images show poor quality SNP data (top) that is improved by preamplifying the samples (bottom). 
Amplification plot is missing expected cluster	Signal saturation caused software to call homozygous alleles as heterozygous (or *vice versa*).	Use real-time traces to manually call alleles. See "Recommended: Run OpenArray™ SNP genotyping experiments in real-time mode" on page 49 for more information.

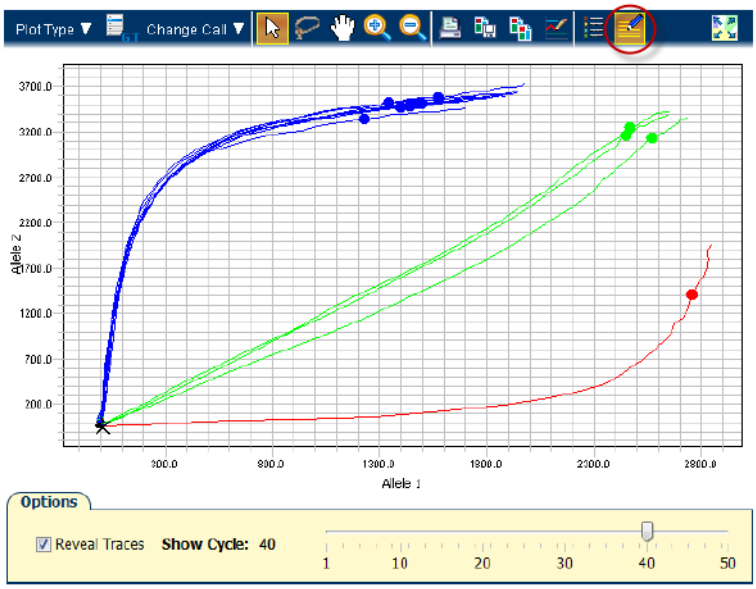
Observation	Possible cause	Recommended action
Incorrect genotype called	Real-time traces were not used to identify outlier data points.	Use real-time traces to identify outlier data points and to manually call alleles. Real-time traces can be viewed in the QuantStudio™ 12K Flex Software or the Connect Genotyping Module, which is accessed from **thermofisher.com/cloud**. See "Recommended: Run OpenArray™ SNP genotyping experiments in real-time mode" on page 49 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 100). 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 103 and “Analysis guidelines: SNP genotyping assays for triallelic SNPs” on page 103 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".

B

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 *Pharmacogenomics Experiments User Guide*.
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" indicates that the material is available from **fisherscientific.com** or another 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, then 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 minutes
Cycling (10 to 14 cycles)	Denature	95°C	15 seconds
	Anneal/Extend	60°C	4 minutes
Hold	Inactivate	99.9°C	10 minutes
Hold	—	4°C	up to 1 hour 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, then 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 `SampleFile_384-Well Plate_192-Format.xlsx` calculates the concentration of test DNA samples with this calibrator method.

Note: The `SampleFile_384-Well Plate_192-Format.xlsx` assumes duplicate RNase P reactions in a 384-well plate layout.

Download the `SampleFile_384-Well Plate_192-Format.xlsx` from **thermofisher.com/oaqrc**.

Required materials

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Cat. No.
TaqMan™ Copy Number Reference Assay, human, RNase P (20X)	4403326
TaqMan™ Control Genomic DNA (human) (ready for use)	4312660
TaqPath™ ProAmp™ Master Mix	A30865

Before you begin

- Dilute test DNA samples 1:10 in nuclease-free water.
- Determine the total number of reactions required by including the following items:
  - 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 `SampleFile_384-Well Plate_192-Format.xlsx` is set up for duplicate reactions.
- Generate the RNase P Sample TXT file from the `SampleFile_384-Well Plate_192-Format.xlsx`.

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	5.0 µL
TaqMan™ Copy Number Reference Assay, human, RNase P (20X)	0.5 µL
Nuclease-free Water	2.5 µL
Total	**8 µL**

[1] See the `SampleFile_384-Well Plate_192-Format.xlsx` 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, TaqMan™ Control Genomic DNA (human), 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 thermal cycling conditions and settings:

Step	Temperature	Time	Cycles
Enzyme activation	95°C	10 minutes	1
Denature	95°C	15 seconds	40
Anneal/Extend	60°C	60 seconds	

   - Experiment type: **Standard Curve**
   - Mode: **Standard**
   - Reporter: **VIC™**
   - Quencher: **TAMRA™**
   - Passive reference: **ROX**

6. To assign the sample names, import the RNase P Sample TXT file generated from the `SampleFile_384-Well Plate_192-Format.xlsx`.

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 `SampleFile_384-Well Plate_192-Format.xlsx`.
    b. Enter the dilution factor of the test DNA samples into the `SampleFile_384-Well Plate_192-Format.xlsx`.

D 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)

wwwn.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:**wwwn.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 17). 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 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 **GeneArtSupport@thermofisher.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 DNA*Zap*™ PCR DNA Degradation Solutions (Cat. No. AM9890).

F

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, and so on). 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 sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer 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 needed) 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.

AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

WARNING! **HAZARDOUS WASTE (from instruments).** Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! **4L Reagent and Waste Bottle Safety.** Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! **Potential Biohazard.** Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

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)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

Documentation and support

Related documentation

Document	Pub. No.
Thermo Scientific™ KingFisher™ Flex User Manual	N07669
MagMAX™ DNA Multi-Sample Ultra Kit MagMAX™ Express-96 Magnetic Particle Processor Protocol	4428202
MagMAX™ DNA Multi-Sample Ultra Kit (whole blood) User Guide	MAN0010294
Best Practices for Collection of Buccal Swabs for Genotyping Experiments	MAN0014348
Isolation of DNA for Genotyping Experiments	MAN0014561
OpenArray™ Sample Tracker Software Quick Reference	4460657
OpenArray™ SNP Genotyping Experiments	MAN0014351
OpenArray™ Genotyping Analysis	MAN0014352
TaqMan™OpenArray™ Genotyping Troubleshooting Guide	MAN0011115
TaqMan™ Genotyper Software Getting Started Guide	4448637
TaqMan™ SNP Genotyping Assays User Guide	MAN0009593
TaqMan™ Drug Metabolism Assays for Triallelic SNPs Application Note	135AP01-01
Copy Number Analysis for Pharmacogenomics Experiments	MAN0014350
RNase P Quantification for Genotyping Experiments	MAN0014349
Creating Standard Curves with Genomic DNA or Plasmid DNA Templates for Use in Quantitative PCR Application Note	4371090
TaqMan™ Copy Number Assays User Guide	4397425
CYP2D6 Allele-specific Copy Number Analysis Quick Reference	MAN0011114
Copy Number Analysis for Pharmacogenomics Experiments	MAN0014350
CopyCaller™ Software v2.0 User Guide	4400042
QuantStudio™ 12K Flex Real–Time PCR System Software Help	—
QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Plate Quick Reference	4478673
QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Experiments User Guide	4470935

(continued)

Document	Pub. No.
QuantStudio™ 12K Flex Real–Time PCR System Maintenance and Administration Guide	4470689
OpenArray™ AccuFill™ System User Guide, for OpenArray™ AccuFill™ Software v1.2	4456986
OpenArray™ Sample Tracker Software Quick Reference, for OpenArray™ AccuFill™ Software v1.2	4460657
QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide, for OpenArray™ AccuFill™ Software v2.0	MAN0025669
OpenArray™ AccuFill™ Software v2.0 Full Run Workflow Quick Reference	MAN0025836
OpenArray™ AccuFill™ Software v2.0 Quick Run Workflow Without Sample Information Quick Reference	MAN0025835

Customer and technical support

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  - 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 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**.

References

Gaedigk A, Bradford LD, Alander SW, and Leeder JS (2006) CYP2D6*36 gene arrangements within the cyp2d6 locus: association of CYP2D6*36 with poor metabolizer status. *Drug Metab Dispos* 3:563-569.

He Y, Hoskins JM, and McLeod HL (2011) Copy number variants in pharmacogenetic genes. *Trends Mol Med* 17:244-251.

Oscarson M, McLellan RA, Asp V, Ledesma M, Bernal Ruiz ML, Sinues B, Rautio A, and Ingelman-Sundberg M (2002) Characterization of a novel CYP2A7/CYP2A6 hybrid allele (CYP2A6*12) that causes reduced CYP2A6 activity. *Hum Mutat* 20:275-283.

Ramamoorthy A, Flockhart DA, Hosono N, Kubo M, Nakamura Y, and Skaar TC (2010) Differential quantification of CYP2D6 gene copy number by four different quantitative real-time PCR assays. *Pharmacogenet Genomics* 20:451-454.

Sim, S.C. and Ingelman-Sundberg, M (2010) The Human Cytochrome P450 (CYP) Allele Nomenclature website: a peer-reviewed database of CYP variants and their associated effects. *Hum Genomics* 4:278–281.

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