

Pharmacogenomics Experiments

User Guide Addendum

For use with:

384-well TaqMan® Array Plates

ProFlex™ PCR System

QuantStudio® 12K Flex System

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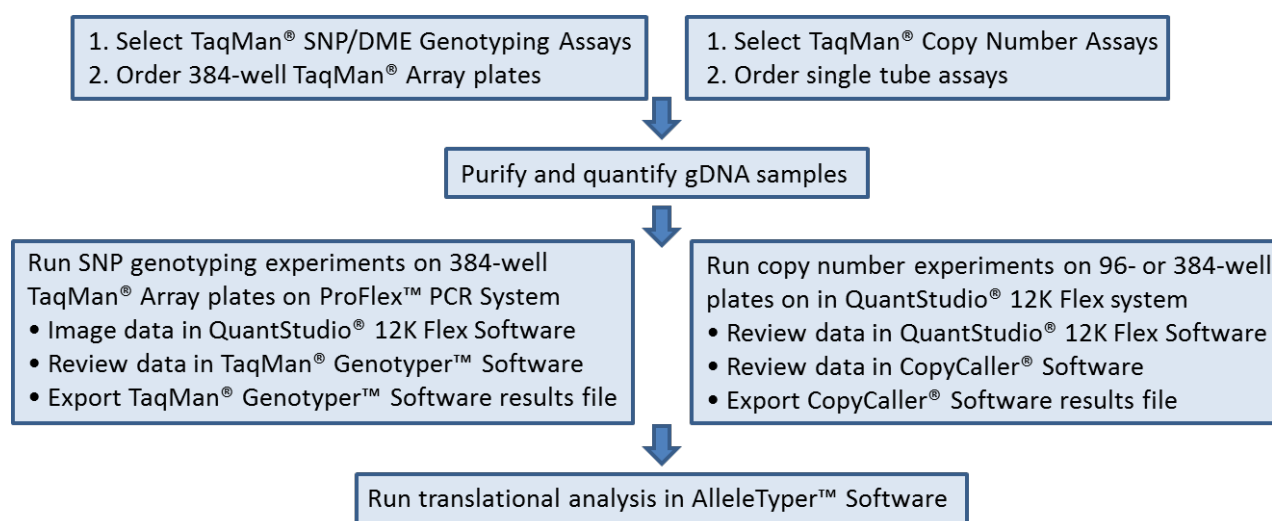
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Chapter 1 About the PGx Workflow

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. The *Pharmacogenomics Experiments User Guide* (Pub. # MAN0009612) describes Life Technologies' complete sample-to result PGx workflow solution using the QuantStudio® 12K Flex Real-Time PCR System and using TaqMan® OpenArray® Genotyping Plates for SNP genotyping. This *Pharmacogenomics Experiments User Guide Addendum* covers the alternative workflow wherein SNP genotyping is performed using 384-well TaqMan® Array Plates run on the ProFlex™ PCR System and imaged on the QuantStudio® 12K Flex Real-Time PCR System.

Workflow



User Guide Contents

This *Pharmacogenomics Experiments User Guide Addendum* covers the following aspects of this workflow:

- Order 384-well TaqMan® Array plates
- Run SNP genotyping experiments on 384-well TaqMan® Array plates on ProFlex™ PCR System
- Image data in QuantStudio® 12K Flex Software

Refer to the *Pharmacogenomics Experiments User Guide* (Pub. # MAN0009612) for information on all other aspects of workflow, including TaqMan assay information and selection, sample preparation, SNP genotyping data analysis, running copy number experiments and copy number data analysis, and translation of genotyping results.

Chapter 2 Ordering information

This chapter covers ordering TaqMan® Array plates. For information on single tube assays and reagents required for PGx genotyping experiments, refer to Chapter 3 in the *Pharmacogenomics Experiments User Guide*.

PGx TaqMan® Array plate products and formats

PGx TaqMan® Array plates are 384-well format plates that contain pre-plated, dried down TaqMan® SNP Genotyping Assays (can contain DME, Predesigned, and/or Custom assays). The following Made-to-Order products are available:

- 384-well TaqMan® Array PGx Express panel plate contains 64 pre-selected, wet-lab validated TaqMan® DME and SNP genotyping assays. Six samples can be run on each plate. The Express panel includes our most popular PGx assay content to targets in the following genes: CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP3A5, SLCO1B1, VKORC1, COMT, OPRM1, DRD2, FII, FV, MTHFR, APOE2, APOE4
- Custom 384-well TaqMan® Array PGx plates enable you to build your own assay panel. Assays can be selected from our entire 6.2 million DME and SNP assay collection. Three different formats are available:

Number of assays	Number of samples
32	12
64	6
128	3

Ordering PGx TaqMan® Array plates

For information on ordering PGx TaqMan® Array plates, please send an email to: QuantStudioFrontDesk@lifetech.com.

PGx TaqMan® Array plate storage conditions

Store the unopened plates at room temperature until the expiration date provided on the package label.

Chapter 3 Prepare, run, and analyze TaqMan® Array PGx plate genotyping experiments

The information provided in this chapter includes streamlined procedures for running 384-well TaqMan® Array PGx plate genotyping experiments on the ProFlex™ PCR System followed by analysis on the QuantStudio™12K Flex Real-Time PCR System. For information on preparing samples for this workflow, and for downstream data analysis using TaqMan® Genotyper™ Software, refer to the *Pharmacogenomics Experiments User Guide* (Pub. # MAN0009612).

About the TaqMan® Array PGx plate Genotyping Protocol

This chapter provides summary procedures for performing experiments described in detail in the *QuantStudio™12K Flex Real-Time PCR System OpenArray® Experiments User Guide* (4470935).

Materials List

Part Number	Vendor	Description	Format 32 Quantity per 48 Samples	Format 64 Quantity per 48 Samples	Format 128 Quantity per 48 Samples
N/A	Life Technologies	384-well TaqMan® Array PGx Plates*	4 plates	8 plates	16 plates
4371357	Life Technologies	TaqMan® Genotyping Master Mix (50 mL)	5 mL	10 mL	20 mL
N/A	N/A	Genomic DNA (MagMax™ sample preparation)	100 µL of 5 ng/µL DNA per sample**	200 µL of 5 ng/µL DNA per sample**	350 µL of 5 ng/µL DNA per sample**
260251	Thermo Scientific	Nunc™ 1.3 mL 96 DeepWell™ Plate, Sterile	1 plate	1 plate	1 plate
4306311	Life Technologies	MicroAmp® Clear Adhesive Film (100 films)	2 films	2 films	2 films
4360954	Life Technologies	MicroAmp® Optical Adhesive Film (25 covers)	4 covers	8 covers	16 covers
4344435	Life Technologies	Reservoir, 20 mL (50 reservoirs)	1 reservoir	1 reservoir	1 reservoir

* Store the unopened plates at room temperature until the expiration date provided on the package label.

** For 12.5 ng DNA/5 µL reaction. DNA amounts from 3 - 12.5 ng/reaction can be used.

DNA samples

For detailed guidance on preparing DNA samples, refer to Chapter 4: *Prepare genomic DNA samples* in the *Pharmacogenomics Experiments User Guide*. We recommend diluting DNA preparations to 5 ng/ μ L stock solutions, which can be used directly in both TaqMan® Array PGx plate genotyping and copy number variation experiments. A 5 ng/ μ L stock concentration will provide 12.5 ng DNA/5 μ L SNP genotyping reaction, which is the amount of DNA that is used in the protocols presented herein. If desired, less DNA can be used: input DNA quantities from 3 - 12.5 ng have been shown to provide high quality genotyping data. Note that the same amount of DNA should be used for each sample and reaction within an experiment for best genotype clustering results.

For proper genotype cluster analysis, it is recommended that one in 24 samples in a PGx experiment is a No Template Control (NTC); i.e. it contains water instead of DNA. For example, in a 48 sample experiment, run 46 gDNA samples and 2 NTCs.

TaqMan® Array PGx plate Genotyping Workflow Overview

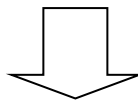
1. Prepare the Sample Source Plate by combining samples and master mix into a 96-well Source Plate. The combined sample and master mix is referred to as the 'reaction mix' throughout this guide.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	4	5	6	7	8	9	10	11	12
B	13	14	15	16	17	18	19	20	21	22	23	24
C	25	26	27	28	29	30	31	32	33	34	35	36
D	37	38	39	40	41	42	43	44	45	46	47	48
E	49	50	51	52	53	54	55	56	57	58	59	60
F	61	62	63	64	65	66	67	68	69	70	71	72
G	73	74	75	76	77	78	79	80	81	82	83	84
H	85	86	87	88	89	90	91	92	93	94	95	96

2. Transfer reaction mixtures into dried down 384-well TaqMan® Array PGx plates. In the example shown, 6 samples are transferred to a 64 assay format 384-well plate.

96-well Sample Source plate

	1	2	3	4	5	6	7
A	1	2	3	4	5	6	7
B	13	14	15	16	17	18	19



384-well TaqMan® Array PGx Plate 1

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	2	1	2	1	2	1	2	1	2	1	2	33	34	33	34	33	34	33	34	33	34	33	34
B	3	4	3	4	3	4	3	4	3	4	3	4	35	36	35	36	35	36	35	36	35	36	35	36
C	5	6	5	6	5	6	5	6	5	6	5	6	37	38	37	38	37	38	37	38	37	38	37	38
D	7	8	7	8	7	8	7	8	7	8	7	8	39	40	39	40	39	40	39	40	39	40	39	40
E	9	10	9	10	9	10	9	10	9	10	9	10	41	42	41	42	41	42	41	42	41	42	41	42
F	11	12	11	12	11	12	11	12	11	12	11	12	43	44	43	44	43	44	43	44	43	44	43	44
G	13	14	13	14	13	14	13	14	13	14	13	14	45	46	45	46	45	46	45	46	45	46	45	46
H	15	16	15	16	15	16	15	16	15	16	15	16	47	48	47	48	47	48	47	48	47	48	47	48
I	17	18	17	18	17	18	17	18	17	18	17	18	49	50	49	50	49	50	49	50	49	50	49	50
J	19	20	19	20	19	20	19	20	19	20	19	20	51	52	51	52	51	52	51	52	51	52	51	52
K	21	22	21	22	21	22	21	22	21	22	21	22	53	54	53	54	53	54	53	54	53	54	53	54
L	23	24	23	24	23	24	23	24	23	24	23	24	55	56	55	56	55	56	55	56	55	56	55	56
M	25	26	25	26	25	26	25	26	25	26	25	26	57	58	57	58	57	58	57	58	57	58	57	58
N	27	28	27	28	27	28	27	28	27	28	27	28	59	60	59	60	59	60	59	60	59	60	59	60
O	29	30	29	30	29	30	29	30	29	30	29	30	61	62	61	62	61	62	61	62	61	62	61	62
P	31	32	31	32	31	32	31	32	31	32	31	32	63	64	63	64	63	64	63	64	63	64	63	64

3. Perform PCR on ProFlex™ PCR System thermal cyclers.
4. Read plates on the QuantStudio™ 12K Instrument.
5. Perform data analysis using TaqMan® Genotyper™ Software.

Format 32: Preparing the 96-well reaction mix source plate

1. Dilute the samples to 5 ng/ μ L (minimum: 100 μ L of each sample).
2. Transfer 100 μ L of the diluted samples into a 1.2 mL 96- well plate.

Note: We recommend that one in every 24 samples is a NTC.

Sample layout in 96-well plate (up to 96 samples)

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	4	5	6	7	8	9	10	11	12
B	13	14	15	16	17	18	19	20	21	22	23	24
C	25	26	27	28	29	30	31	32	33	34	35	36
D	37	38	39	40	41	42	43	44	45	46	47	48
E	49	50	51	52	53	54	55	56	57	58	59	60
F	61	62	63	64	65	66	67	68	69	70	71	72
G	73	74	75	76	77	78	79	80	81	82	83	84
H	85	86	87	88	89	90	91	92	93	94	95	96

3. Add 100 μ L of 2x TaqMan® Genotyping Master Mix into the sample-containing wells of the 96-well plate.
4. Seal the plate with an adhesive cover, gently vortex for 5 seconds, and centrifuge at 1000 rpm for 1 minute.

Format 32: Transferring reaction mix into 384-well PGx plates

Thirty-two (32) assays are dried down in the 384-well plates in the format below: 32 assays are replicated 12 times in each plate. Each 384-well PGx plate can be used to test up to 12 samples. Therefore 4 PGx plates are needed to test 48 samples and 8 PGx plates are needed to test 96 samples.

Note: We strongly recommend using an electronic 12-channel pipette to simplify this portion of the workflow.

Format 32 Assay layout for 384-well PGx Plate

	Set 1		Set 2		Set 3		Set 4		Set 5		Set 6		Set 7		Set 8		Set 9		Set 10		Set 11		Set 12	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
B	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4
C	5	6	5	6	5	6	5	6	5	6	5	6	5	6	5	6	5	6	5	6	5	6	5	6
D	7	8	7	8	7	8	7	8	7	8	7	8	7	8	7	8	7	8	7	8	7	8	7	8
E	9	10	9	10	9	10	9	10	9	10	9	10	9	10	9	10	9	10	9	10	9	10	9	10
F	11	12	11	12	11	12	11	12	11	12	11	12	11	12	11	12	11	12	11	12	11	12	11	12
G	13	14	13	14	13	14	13	14	13	14	13	14	13	14	13	14	13	14	13	14	13	14	13	14
H	15	16	15	16	15	16	15	16	15	16	15	16	15	16	15	16	15	16	15	16	15	16	15	16
I	17	18	17	18	17	18	17	18	17	18	17	18	17	18	17	18	17	18	17	18	17	18	17	18
J	19	20	19	20	19	20	19	20	19	20	19	20	19	20	19	20	19	20	19	20	19	20	19	20
K	21	22	21	22	21	22	21	22	21	22	21	22	21	22	21	22	21	22	21	22	21	22	21	22
L	23	24	23	24	23	24	23	24	23	24	23	24	23	24	23	24	23	24	23	24	23	24	23	24
M	25	26	25	26	25	26	25	26	25	26	25	26	25	26	25	26	25	26	25	26	25	26	25	26
N	27	28	27	28	27	28	27	28	27	28	27	28	27	28	27	28	27	28	27	28	27	28	27	28
O	29	30	29	30	29	30	29	30	29	30	29	30	29	30	29	30	29	30	29	30	29	30	29	30
P	31	32	31	32	31	32	31	32	31	32	31	32	31	32	31	32	31	32	31	32	31	32	31	32

1. Remove the foil wrap and heat seal from a 384-well PGx plate.
2. Program the electronic pipette tip to perform 32, repeated 5 μ L dispenses.

3. Load 12 new tips onto an electronic 12-channel pipette and aspirate from wells A1 through A12 from the 96-well source plate.
4. Transfer 5 μ L of the reaction mixes into the corresponding wells of the PGx plate according to the layout below:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B																								
C																								
D																								
E																								
F																								
G																								
H																								
I																								
J																								
K																								
L																								
M																								
N																								
O																								
P																								

5. Eject the tips into the waste.
6. Seal the plate with an optical adhesive seal.
7. Vigorously vortex the plate for 5 seconds to ensure proper mixing of the dried down assay with the reaction mixture.
8. Centrifuge at 1000 rpm for 1 minute.
9. Repeat for the remaining samples.

Format 64: Preparing the 96-well reaction mix source plate

1. Dilute the samples to 5 ng/ μ L (minimum: 200 μ L of each sample).
2. Transfer 200 μ L of the diluted samples into a 1.2 mL 96- well plate.

Note: We recommend that one in every 24 samples is a NTC.

Sample layout in 96-well plate (up to 96 samples)

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	4	5	6	7	8	9	10	11	12
B	13	14	15	16	17	18	19	20	21	22	23	24
C	25	26	27	28	29	30	31	32	33	34	35	36
D	37	38	39	40	41	42	43	44	45	46	47	48
E	49	50	51	52	53	54	55	56	57	58	59	60
F	61	62	63	64	65	66	67	68	69	70	71	72
G	73	74	75	76	77	78	79	80	81	82	83	84
H	85	86	87	88	89	90	91	92	93	94	95	96

3. Add 200 μ L of 2x TaqMan® Genotyping Master Mix into the sample-containing wells of the 96-well plate.
4. Seal the plate with an adhesive cover, gently vortex for 5 seconds, and centrifuge at 1000 rpm for 1 minute.

Format 64: Transferring reaction mix into 384-well PGx plates

Sixty-four (64) assays are dried down in the 384-well plates in the format below: assays 1 through 32 are dried down (6 replicates) in the left half of the plate and the remaining 32 assays are dried down on the right half. Each 384-well PGx plate can be used to test up to 6 samples. Therefore 8 PGx plates are needed to test 48 samples and 16 PGx plates are needed to test 96 samples.

Note: We strongly recommend using an electronic 12-channel pipette to simplify this portion of the workflow.

Format 64 Assay layout for 384-well PGx Plate

	Set 1a		Set 2a		Set 3a		Set 4a		Set 5a		Set 6a		Set 1b		Set 2b		Set 3b		Set 4b		Set 5b		Set 6b	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	2	1	2	1	2	1	2	1	2	1	2	33	34	33	34	33	34	33	34	33	34	33	34
B	3	4	3	4	3	4	3	4	3	4	3	4	35	36	35	36	35	36	35	36	35	36	35	36
C	5	6	5	6	5	6	5	6	5	6	5	6	37	38	37	38	37	38	37	38	37	38	37	38
D	7	8	7	8	7	8	7	8	7	8	7	8	39	40	39	40	39	40	39	40	39	40	39	40
E	9	10	9	10	9	10	9	10	9	10	9	10	41	42	41	42	41	42	41	42	41	42	41	42
F	11	12	11	12	11	12	11	12	11	12	11	12	43	44	43	44	43	44	43	44	43	44	43	44
G	13	14	13	14	13	14	13	14	13	14	13	14	45	46	45	46	45	46	45	46	45	46	45	46
H	15	16	15	16	15	16	15	16	15	16	15	16	47	48	47	48	47	48	47	48	47	48	47	48
I	17	18	17	18	17	18	17	18	17	18	17	18	49	50	49	50	49	50	49	50	49	50	49	50
J	19	20	19	20	19	20	19	20	19	20	19	20	51	52	51	52	51	52	51	52	51	52	51	52
K	21	22	21	22	21	22	21	22	21	22	21	22	53	54	53	54	53	54	53	54	53	54	53	54
L	23	24	23	24	23	24	23	24	23	24	23	24	55	56	55	56	55	56	55	56	55	56	55	56
M	25	26	25	26	25	26	25	26	25	26	25	26	57	58	57	58	57	58	57	58	57	58	57	58
N	27	28	27	28	27	28	27	28	27	28	27	28	59	60	59	60	59	60	59	60	59	60	59	60
O	29	30	29	30	29	30	29	30	29	30	29	30	61	62	61	62	61	62	61	62	61	62	61	62
P	31	32	31	32	31	32	31	32	31	32	31	32	63	64	63	64	63	64	63	64	63	64	63	64

1. Remove the foil wrap and heat seal from a 384-well PGx plate.
2. Program the electronic pipette tip to perform 32, repeated 5 μ L dispenses. Two aspirates will be needed to fill the entire plate.

3. Load 6 new tips onto the first 6 channels of an electronic multi-channel pipette and aspirate from wells A1 through A6 from the 96-well source plate.
4. Transfer 5 μ L of the reaction mixes into the corresponding wells of the PGx plate according to the layout below:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6		A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6	
B	A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6		A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6	
C	A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6		A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6	
D	A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6		A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6	
E	A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6		A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6	
F	A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6		A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6	
G	A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6		A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6	
H	A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6		A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6	
I	A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6		A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6	
J	A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6		A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6	
K	A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6		A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6	
L	A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6		A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6	
M	A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6		A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6	
N	A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6		A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6	
O	A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6		A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6	
P	A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6		A1: Sample 1		A2: Sample 2		A3: Sample 3		A4: Sample 4		A5: Sample 5		A6: Sample 6	

5. Eject the tips into the waste.
6. Seal the plate with an optical adhesive seal.
7. Vigorously vortex the plate for 5 seconds to ensure proper mixing of the dried down assay with the reaction mixture.
8. Centrifuge at 1000 rpm for 1 minute.
9. Repeat for the remaining samples.

Format 128: Preparing the 96-well reaction mix source plate

1. Dilute the samples to 5 ng/ μ L (minimum: 350 μ L of each sample).
2. Transfer 350 μ L of the diluted samples into a 1.2 mL 96- well plate.

Note: we recommend that one in every 24 samples is a NTC.

Sample layout in 96-well plate (up to 96 samples)

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	4	5	6	7	8	9	10	11	12
B	13	14	15	16	17	18	19	20	21	22	23	24
C	25	26	27	28	29	30	31	32	33	34	35	36
D	37	38	39	40	41	42	43	44	45	46	47	48
E	49	50	51	52	53	54	55	56	57	58	59	60
F	61	62	63	64	65	66	67	68	69	70	71	72
G	73	74	75	76	77	78	79	80	81	82	83	84
H	85	86	87	88	89	90	91	92	93	94	95	96

3. Add 350 μ L of 2x TaqMan® Genotyping Master Mix into the sample-containing wells of the 96-well plate.
4. Seal the plate with an adhesive cover, gently vortex for 5 seconds, and centrifuge at 1000 rpm for 1 minute.

Format 128: Transferring reaction mix into 384-well PGx plates

One hundred twenty-eight (128) assays are dried down in the 384-well plates in the format below. Each 384-well PGx plate can be used to test up to 3 samples. Therefore, 16 PGx plates are needed to test 48 samples and 32 PGx plates are needed to test 96 samples.

Note: We strongly recommend using an electronic 12-channel pipette to simplify this portion of the workflow.

Format 128 Assay layout for 384-well PGx Plate

	Set 1a		Set 2a		Set 3a		Set 1b		Set 2b		Set 3b		Set 1c		Set 2c		Set 3c		Set 1d		Set 2d		Set 3d	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1 A	1	2	1	2	1	2	33	34	33	34	33	34	65	66	65	66	65	66	97	98	97	98	97	98
2 B	3	4	3	4	3	4	35	36	35	36	35	36	67	68	67	68	67	68	99	100	99	100	99	100
3 C	5	6	5	6	5	6	37	38	37	38	37	38	69	70	69	70	69	70	101	102	101	102	101	102
4 D	7	8	7	8	7	8	39	40	39	40	39	40	71	72	71	72	71	72	103	104	103	104	103	104
5 E	9	10	9	10	9	10	41	42	41	42	41	42	73	74	73	74	73	74	105	106	105	106	105	106
6 F	11	12	11	12	11	12	43	44	43	44	43	44	75	76	75	76	75	76	107	108	107	108	107	108
7 G	13	14	13	14	13	14	45	46	45	46	45	46	77	78	77	78	77	78	109	110	109	110	109	110
8 H	15	16	15	16	15	16	47	48	47	48	47	48	79	80	79	80	79	80	111	112	111	112	111	112
9 I	17	18	17	18	17	18	49	50	49	50	49	50	81	82	81	82	81	82	113	114	113	114	113	114
10 J	19	20	19	20	19	20	51	52	51	52	51	52	83	84	83	84	83	84	115	116	115	116	115	116
11 K	21	22	21	22	21	22	53	54	53	54	53	54	85	86	85	86	85	86	117	118	117	118	117	118
12 L	23	24	23	24	23	24	55	56	55	56	55	56	87	88	87	88	87	88	119	120	119	120	119	120
13 M	25	26	25	26	25	26	57	58	57	58	57	58	89	90	89	90	89	90	121	122	121	122	121	122
14 N	27	28	27	28	27	28	59	60	59	60	59	60	91	92	91	92	91	92	123	124	123	124	123	124
15 O	29	30	29	30	29	30	61	62	61	62	61	62	93	94	93	94	93	94	125	126	125	126	125	126
16 P	31	32	31	32	31	32	63	64	63	64	63	64	95	96	95	96	95	96	127	128	127	128	127	128

1. Remove the foil wrap and heat seal from a 384-well PGx plate.
2. Program the electronic pipette tip to perform 32, repeated 5 μ L dispenses. Four aspirates will be needed to fill the entire plate.

3. Load 3 new tips onto the first 3 channels of an electronic multi-channel pipette and aspirate from wells A1 through A3 from the 96-well source plate.
4. Transfer 5 μ L of the reaction mixes into the corresponding wells of the PGx plate according to the layout below:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24			
A	A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3		
B	A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3		
C	A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3		
D	A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3		
E	A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3		
F	A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3		
G	A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3		
H	A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3		
I	A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3		
J	A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3		
K	A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3		
L	A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3		
M	A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3		
N	A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3		
O	A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3		
P	A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3			A1: Sample 1			A2: Sample 2			A3: Sample 3		

5. Eject the tips into the waste.
6. Seal the plate with an optical adhesive seal.
7. Vigorously vortex the plate for 5 seconds to ensure proper mixing of the dried down assay with the reaction mixture.
8. Centrifuge at 1000 rpm for 1 minute.
9. Repeat for the remaining samples.

Thermal Cycling

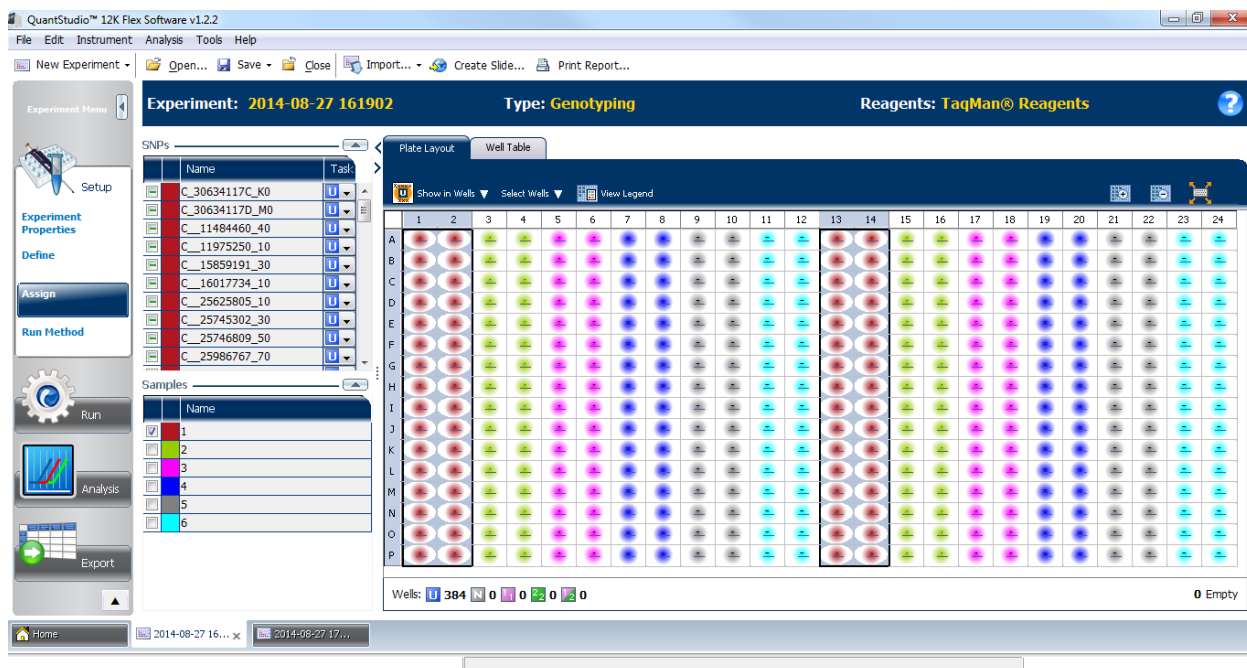
1. Program the ProFlex™ PCR System thermal cyclers with the following PCR run protocol:

Step	Temperature	Duration	Cycles
Enzyme Activation	95° C	10 minutes	HOLD
Denaturation	95° C	15 sec	50 cycles
Annealing/ Extension	60° C	90 sec	
Post-PCR Hold	4° C	∞	HOLD

2. Load the plates on the thermal cyclers. Close the lid and start the runs.
3. The runs should take approximately 2 hours and 10 minutes to complete.

Creating .eds files for the QuantStudio™ 12K Flex Instrument

1. Open the Quantstudio™ 12K Flex Software.
2. Create a New Experiment.
3. Enter your Experiment Name and any additional information in the provided boxes
4. Select 384-Well, Genotyping, TaqMan Reagents, and Standard from the block, experiment, reagents, and properties categories.
5. Deselect the Pre-PCR Read and Amplification boxes at the bottom.
6. Click on Import Plate Setup (top of the window) and browse for the Plate setup file that you created for your TaqMan® Array PGx plate (using the provided 384-well Plate setup file macro).
7. Select the file and click on Start Import. This will populate and assign the assays and samples to the 384-well plate.
8. Click on the Assign tab and ensure that the samples have been correctly assigned to the plate. In this example, 6 sample names have been added for a format 64 assay plate.



9. Click on the Run Method tab. Enter 5 μ L for the Reaction Volume and ensure that only a Post-Read Stage is programmed at 60° C for 30 seconds.
10. Save the file and proceed to create .eds files for the remainder of the plates.

Reading the plates on the QuantStudio™ 12K Flex Instrument

1. Once thermal cycling has completed, remove the plates from the thermal cyclers and centrifuge at 1000 rpm for 1 minute.
2. Load a plate onto the QuantStudio™ 12K Flex instrument.
3. Open the corresponding .eds file for the plate.
4. Click on the Run tab (left window pane).
5. Once the read is complete, click Analysis, then save and close the file.
6. Repeat for the remaining plates.

IMPORTANT! Plates should be read within 12 hours after running to avoid possible sample signal drift and genotyping cluster spread.

Perform analysis in TaqMan® Genotyper™ Software

The *Pharmacogenomics Experiments User Guide* Chapter 5 provides summary information for downstream analysis of PGx genotyping experiments using TaqMan® Genotyper™ Software. The information below is specific to TaqMan® Array PGx plate experiments.

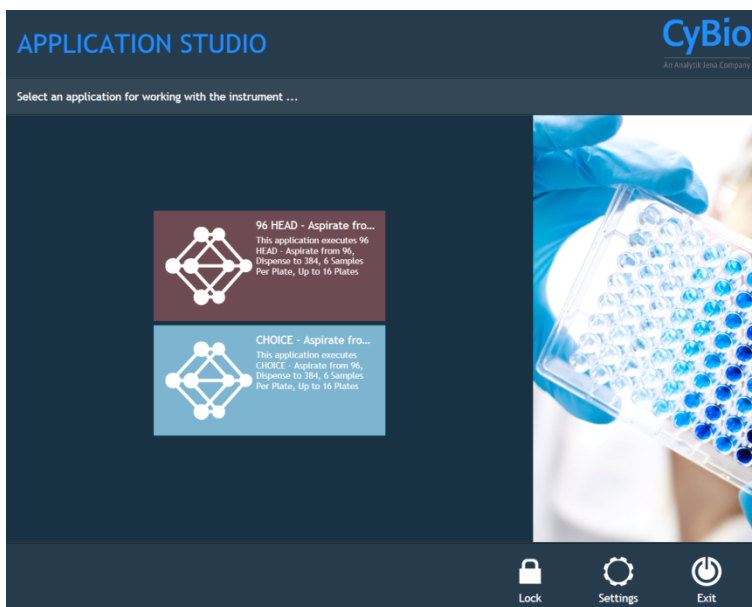
- When creating a study in TaqMan® Genotyper™ Software for PGx experiments performed on 384-well TaqMan® Array Plates run on ProFlex™ PCR System thermal cyclers, select 'Endpoint' from the Experiment Type drop-down menu.

- Import QuantStudio™ 12K Flex .eds files into TaqMan® Genotyper™ Software for genotype analysis.
 - A minimum of 24 samples, including 1 NTC, should be used in a genotyping study for proper genotype clustering.
 - Experiments within a study should have been run on same instrument type (e.g. all ProFlex runs; do not combine ProFlex and QS12k runs).
 - To minimize experimental variation between plates that will be overlaid in a study, use the same lot of Genotyping Master Mix and the same DNA concentration per sample.

Appendix A Plating using the CyBio®-FeliX liquid handling system

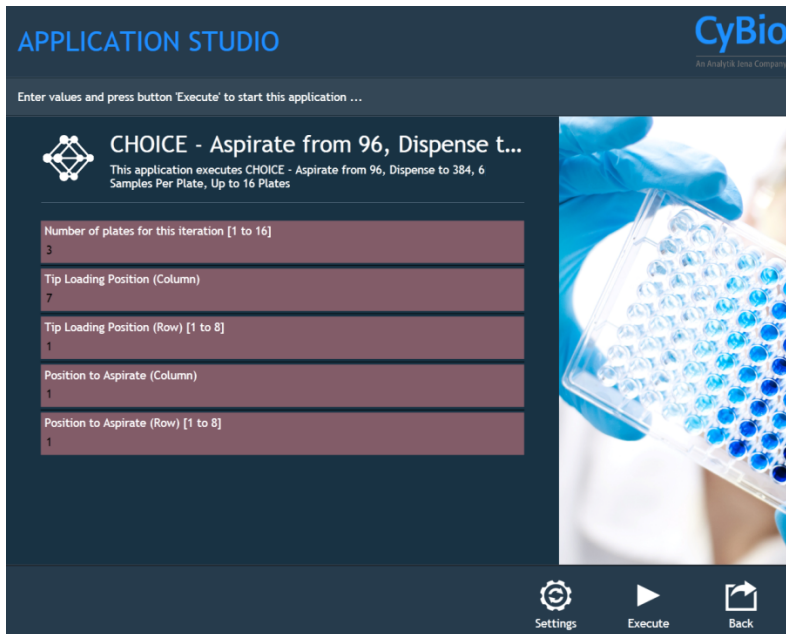
A liquid handling system can be used to facilitate the reaction mix plating procedures described in Chapter 2. This appendix describes a semi-automated process to fill 384-well TaqMan® Array PGx plates using the CyBio®-FeliX liquid handling system, which can be programmed to fill from 1 to 16 plates at a time.

1. Launch the CyBio® Application Studio software.
2. Select the appropriate program based on the head type, then double-click:
 - a. 96 Dispensing Head with 12-Tip Adapter
 - b. CHOICE™ Head with 12-Tip Adapter



Note: If the instrument has just one dispensing head, then only the respective program will be available.

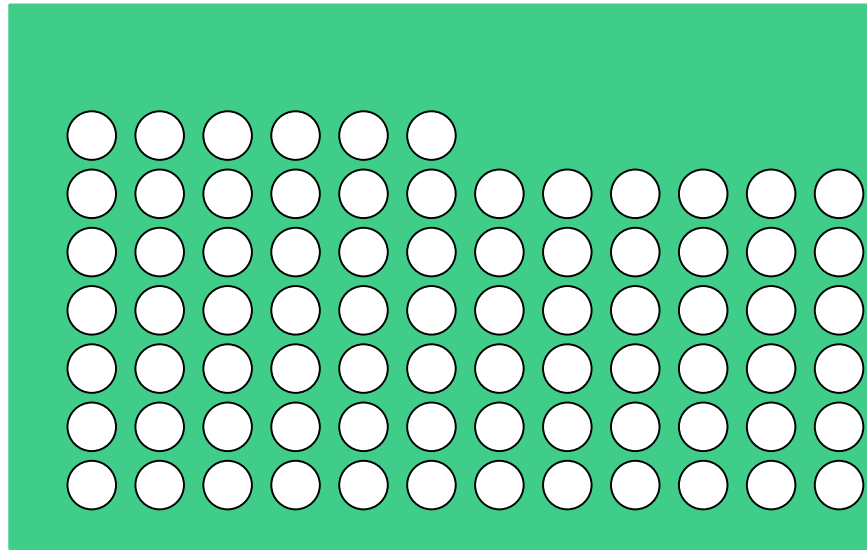
3. At the program screen, there will be prompts to enter values in for the number of plates as well as tip and sample locations.



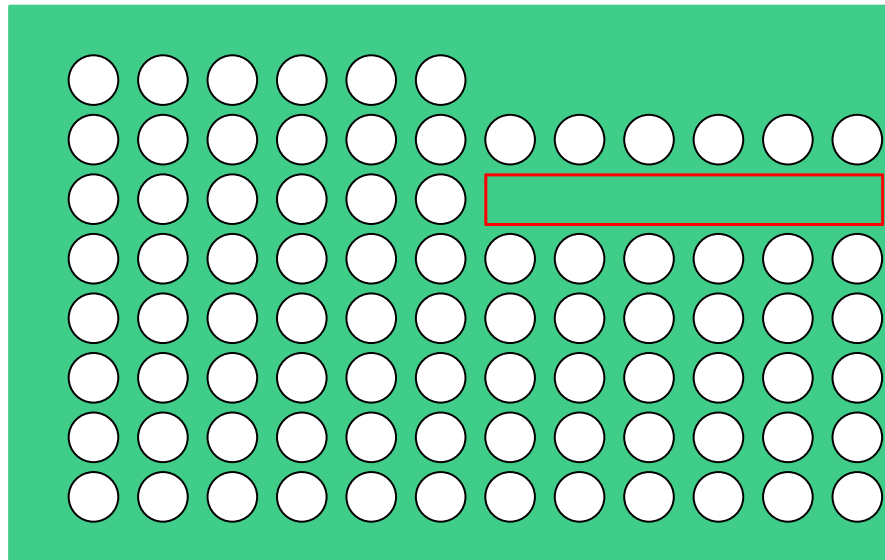
- a. Enter in a value between 1 and 16 for the number of plates to be filled.
- b. Examine the tip box to be used. The instrument takes up 6 tips at a time from RIGHT to LEFT, TOP to BOTTOM. For instance, with a full tip box and 16 plate run, the tips would be used as follows (the numbers indicate which plate the tips would be used for):

02	02	02	02	02	02	01	01	01	01	01	01
04	04	04	04	04	04	03	03	03	03	03	03
06	06	06	06	06	06	05	05	05	05	05	05
08	08	08	08	08	08	07	07	07	07	07	07
10	10	10	10	10	10	09	09	09	09	09	09
12	12	12	12	12	12	11	11	11	11	11	11
14	14	14	14	14	14	13	13	13	13	13	13
16	16	16	16	16	16	15	15	15	15	15	15

Although partially filled tip boxes may be used to fill plates, the tips must be set in the appropriate order, such as in the examples below:

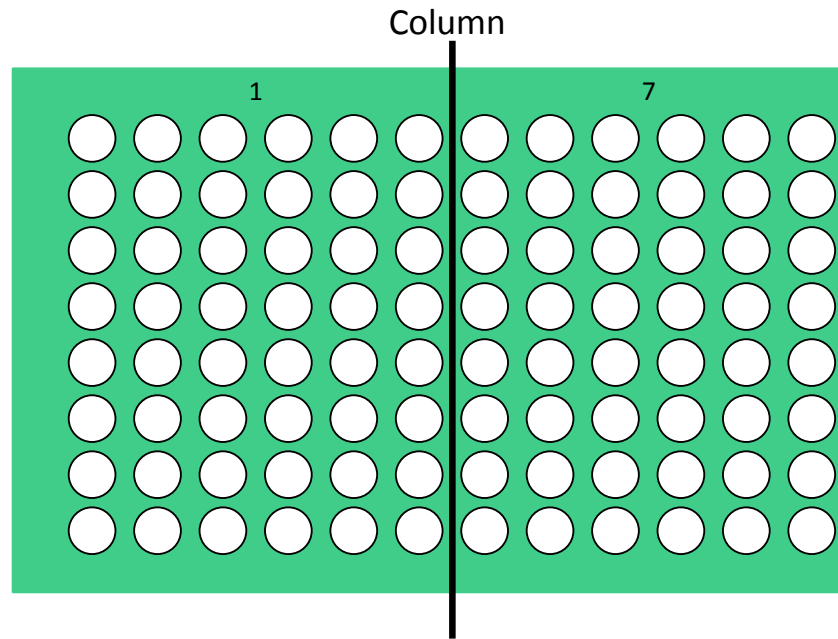


The tip box above shows 3 sections missing; the remaining tips may be used to fill 13 more plates.

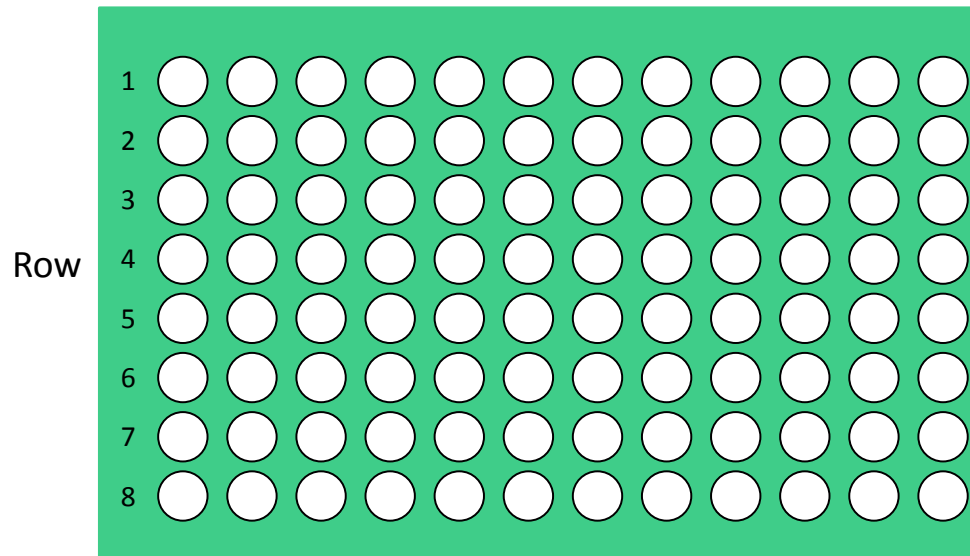


The tip box above shows a missing section that is not in order (highlighted in red). In this case, the instrument cannot be programmed to fill a maximum of 14 plates in a single iteration without physically rearranging the tips to the correct order. This occurrence is highly unlikely during routine operation of the instrument.

- c. Enter the appropriate value for the Tip Loading Position (Column): 1 for the left side of the tip box, or 7 for the right side.



- d. Enter the appropriate value for the Tip Loading Position (Row): 1 – 8



- e. Examine the Reaction Mix Source Plate prepared in the previous section. The instrument takes up 6 reaction samples at a time from LEFT to RIGHT, TOP to BOTTOM to dispense across a 384-well TaqMan® Array plate. For instance, with a full Reaction Mix Source Plate and 16 plate run, the reaction samples would be used as follows (the numbers indicate which plate the samples would be used for):

01	01	01	01	01	01	02	02	02	02	02	02
03	03	03	03	03	03	04	04	04	04	04	04
05	05	05	05	05	05	06	06	06	06	06	06
07	07	07	07	07	07	08	08	08	08	08	08
09	09	09	09	09	09	10	10	10	10	10	10
11	11	11	11	11	11	12	12	12	12	12	12
13	13	13	13	13	13	14	14	14	14	14	14
15	15	15	15	15	15	16	16	16	16	16	16

Although partially filled Reaction Mix Source Plates may be used, the samples must be set in order, such as the example below:

						04	04	04	04	04	04
05	05	05	05	05	05	06	06	06	06	06	06
07	07	07	07	07	07	08	08	08	08	08	08
09	09	09	09	09	09	10	10	10	10	10	10
11	11	11	11	11	11	12	12	12	12	12	12
13	13	13	13	13	13	14	14	14	14	14	14
15	15	15	15	15	15	16	16	16	16	16	16

The example above shows 3 sets of samples missing, and the remaining samples may be used to fill 13 more plates (plates 4 – 16).

01	01	01	01	01	01	02	02	02	02	02	02
03	03	03	03	03	03	04	04	04	04	04	04
05	05	05	05	05	05						
07	07	07	07	07	07	08	08	08	08	08	08
09	09	09	09	09	09	10	10	10	10	10	10
11	11	11	11	11	11	12	12	12	12	12	12
13	13	13	13	13	13	14	14	14	14	14	14
15	15	15	15	15	15	16	16	16	16	16	16

The Reaction Mix Source Plate above shows a sample section 6 missing (highlighted in red) while sample sections 1 – 5 are still available. In this case, the instrument cannot be programmed to fill a maximum 15 plates in a single iteration. The operator must run the program to fill the first 5 plates (shown below highlighted in blue) then run another program to fill the remaining 10 plates (shown below highlighted in green).

01	01	01	01	01	01	02	02	02	02	02	02
03	03	03	03	03	03	04	04	04	04	04	04
05	05	05	05	05	05						
07	07	07	07	07	07	08	08	08	08	08	08
09	09	09	09	09	09	10	10	10	10	10	10
11	11	11	11	11	11	12	12	12	12	12	12
13	13	13	13	13	13	14	14	14	14	14	14
15	15	15	15	15	15	16	16	16	16	16	16

f. Enter the appropriate value for the Position to Aspirate (Column): 1 or 7

Column

1	7
○ ○ ○ ○ ○ ○	○ ○ ○ ○ ○ ○
○ ○ ○ ○ ○ ○	○ ○ ○ ○ ○ ○
○ ○ ○ ○ ○ ○	○ ○ ○ ○ ○ ○
○ ○ ○ ○ ○ ○	○ ○ ○ ○ ○ ○
○ ○ ○ ○ ○ ○	○ ○ ○ ○ ○ ○
○ ○ ○ ○ ○ ○	○ ○ ○ ○ ○ ○
○ ○ ○ ○ ○ ○	○ ○ ○ ○ ○ ○
○ ○ ○ ○ ○ ○	○ ○ ○ ○ ○ ○

g. Enter the appropriate value for the Position to Aspirate (Row): 1 – 8

1	○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○
2	○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○
3	○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○
4	○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○
5	○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○
6	○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○
7	○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○
8	○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○

Row

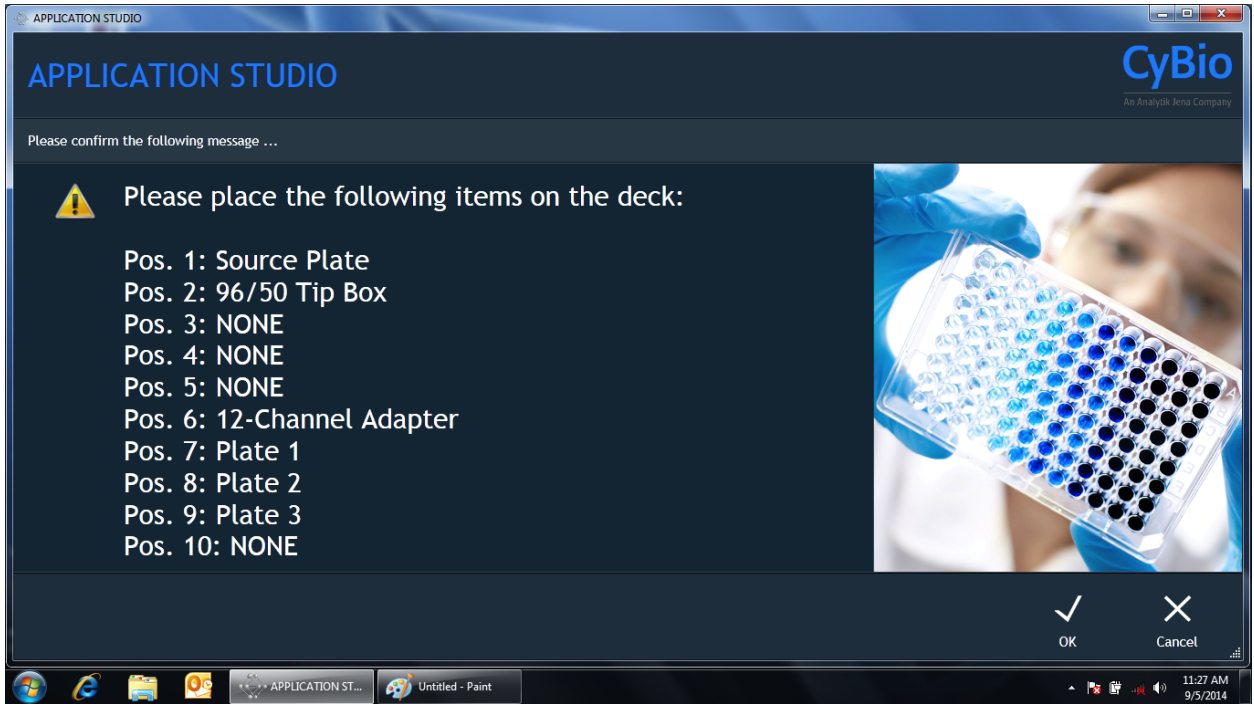
- When the appropriate parameters have been entered, click Execute.



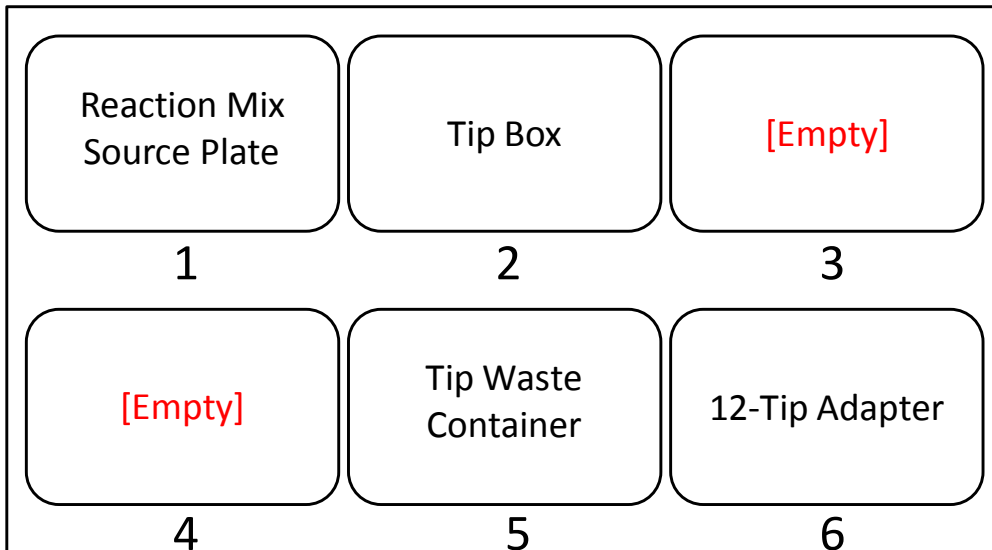
- Confirm by clicking the OK button.



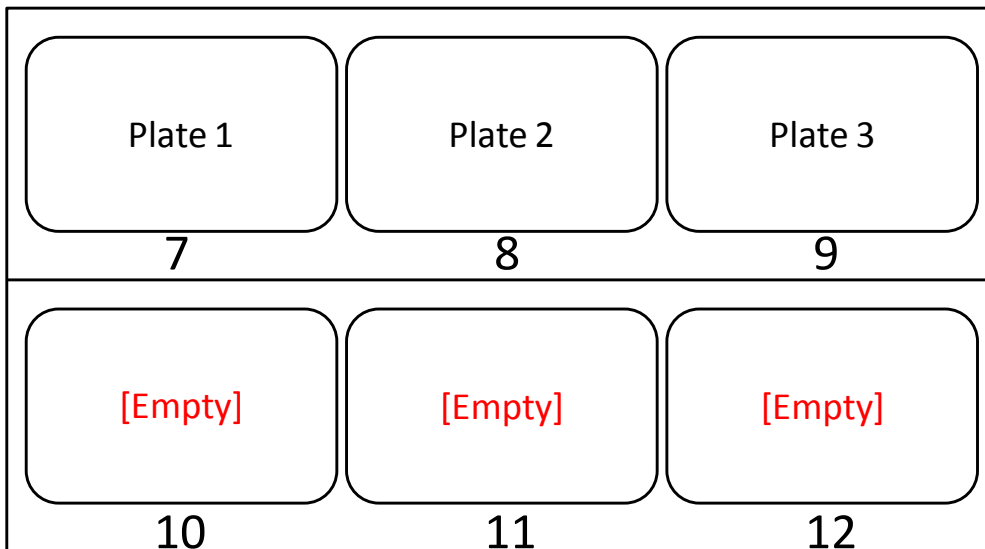
6. Open the instrument door and place the items the deck:



Bottom Level:

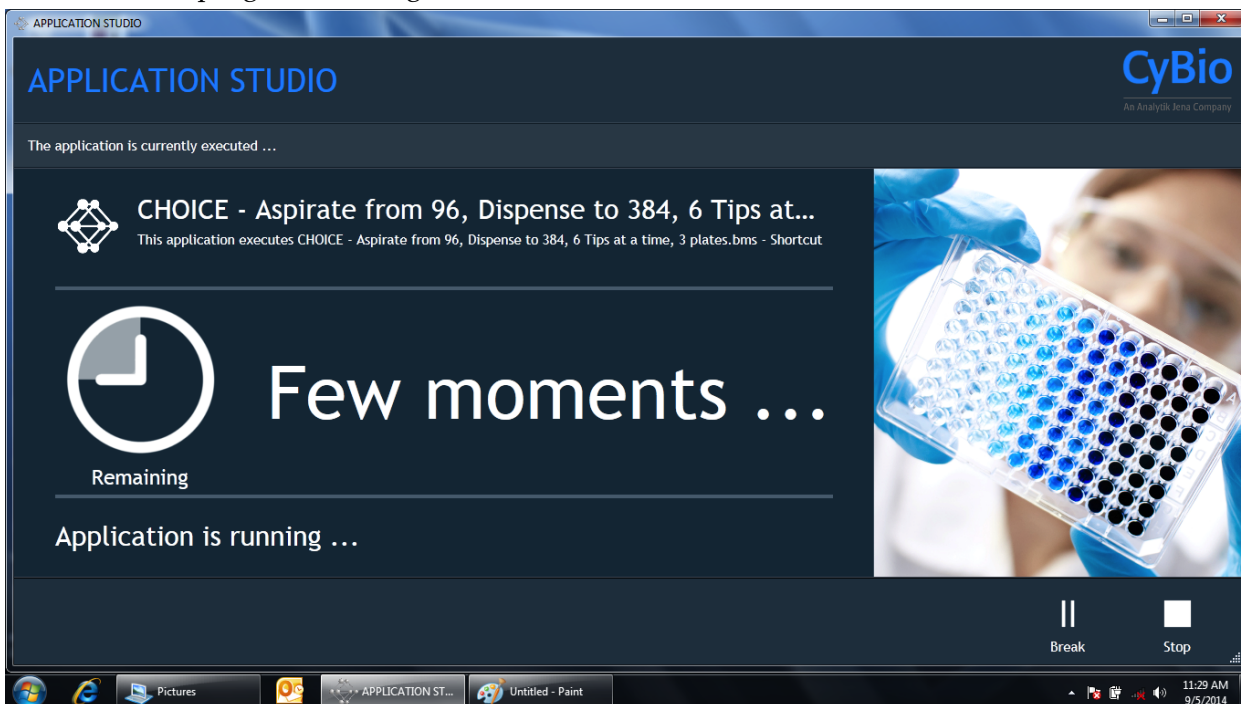


Top Level:

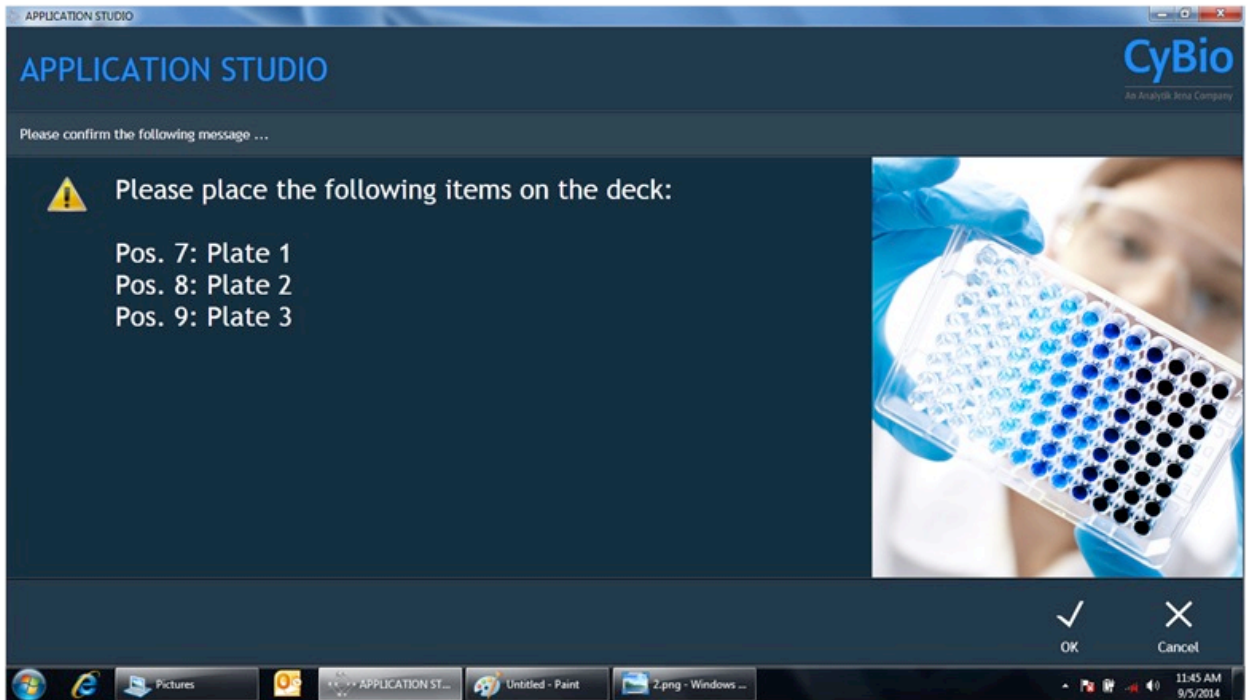


Note: The instrument will dispense into 3 plates at a time then pause to allow the user to remove the filled plates, and reload with up to 3 new plates.

7. Click OK. The program will begin.



8. When the instrument pauses, open the instrument door, remove the filled 384-well TaqMan® Array plates and seal with an optical seal. Vortex the plates at speed 10 for 20 seconds or until the “red dot” of dried assay is suspended in solution. Then centrifuge the plates at 1000 rpm x 1 minute.
9. Reload the instrument deck with up to 3 new 384-well dried-down assay plates, close the instrument door, then click OK.



10. Repeat Steps 8 and 9 until all the plates have been filled.