



MicroRNA Profiling with TaqMan[®] OpenArray[®] Plates



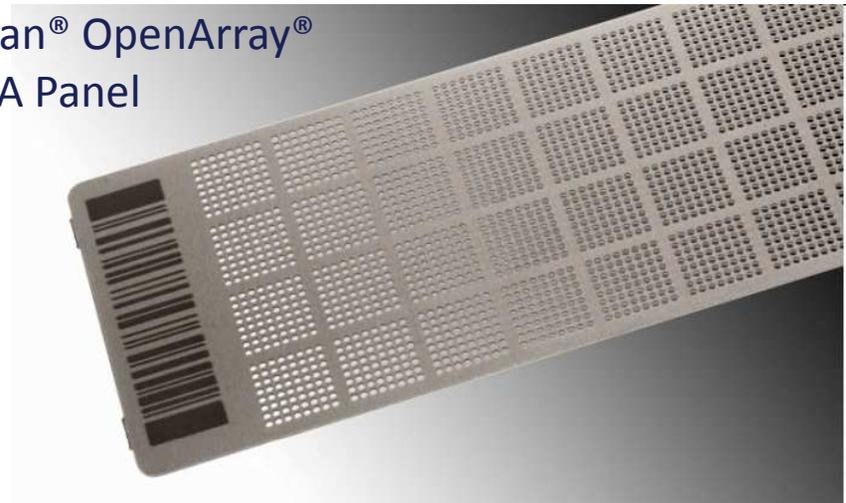
Profiling with TaqMan[®] MicroRNA Assays

Highly Parallel Assays For miRNA Expression Analysis

Megaplex[™]
Primer Pools



TaqMan[®] OpenArray[®]
miRNA Panel



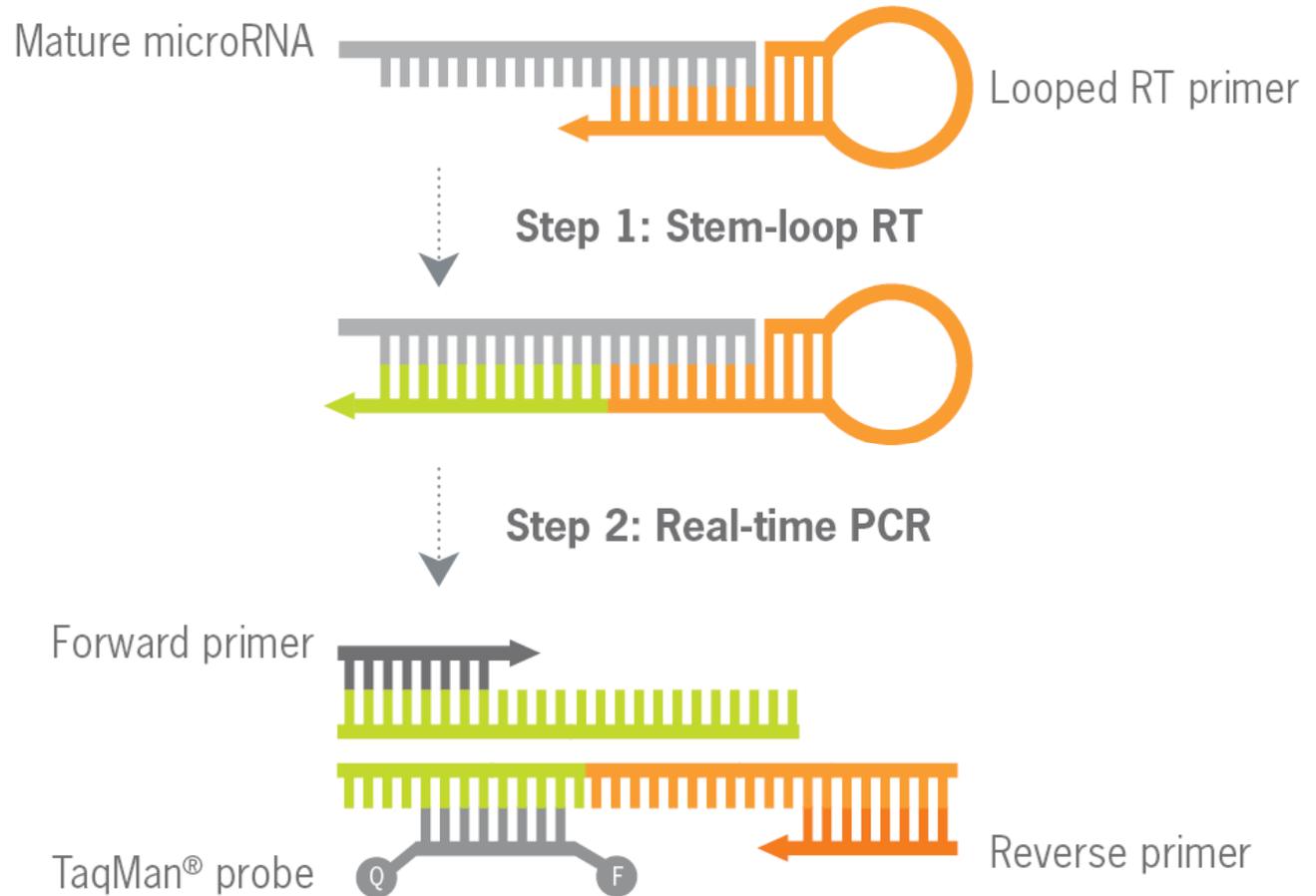
OpenArray[®]
Instrumentation



TaqMan[®] MicroRNA Assays Design

Convert to cDNA

Quantitate
microRNA
Expression

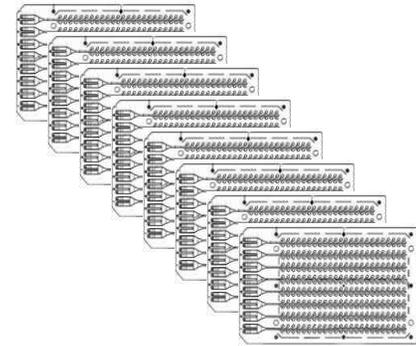


MicroRNA QuantStudio™ 12K Flex Panels

A new standard in high-throughput profiling

What are they?

- Research use, fixed-content OpenArray® MicroRNA Panels containing validated TaqMan® MicroRNA Assays* for Human and Rodent (Mouse and Rat)



Where did they come from?

- Sanger miRBase release v.14.0 (Human) and release v.15.0 (Rodent)

What will they do?

- Profile well-characterized and well-studied miRNAs for Cancer biomarker discovery research, MicroRNA regulation of mRNA gene expression, and MicroRNA functional analysis

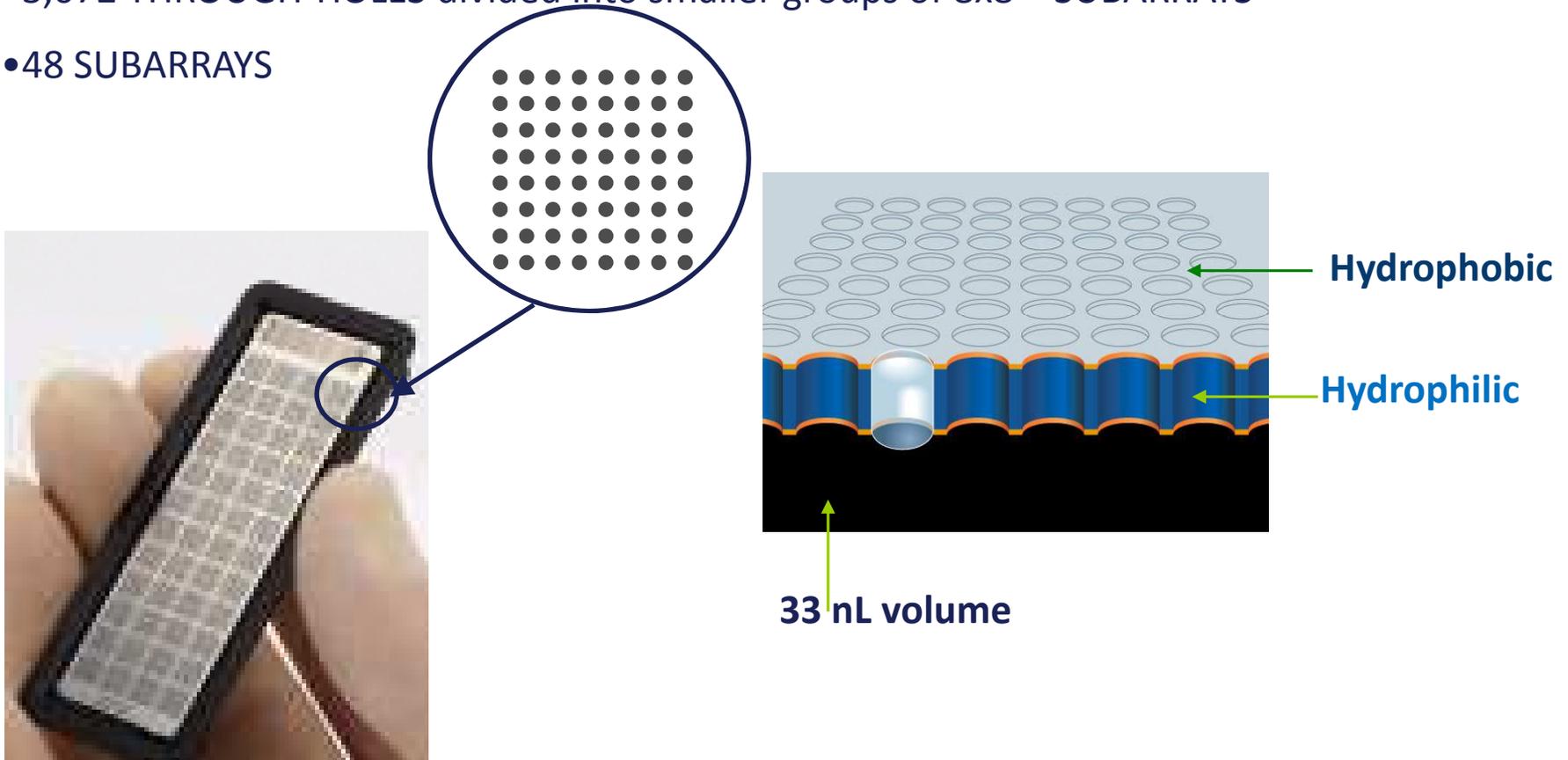
Performance

- >95% of test assays demonstrating StDev <0.5 among replicates



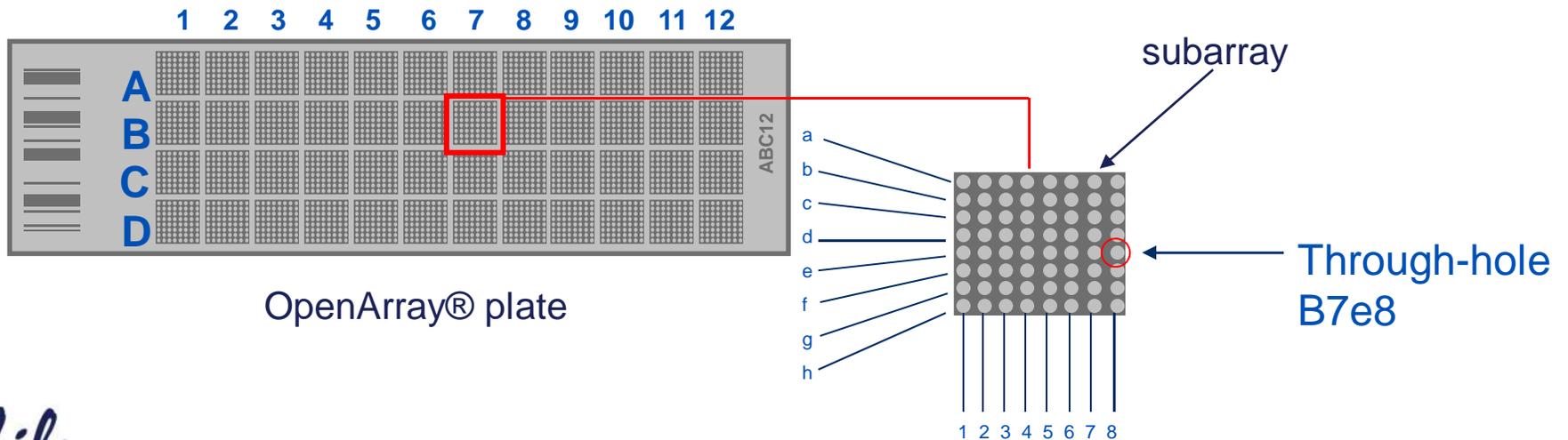
OpenArray[®] plate Anatomy

- OpenArray[®] plate = a microscope slide–sized plate containing 3072 through-holes
- 3,072 THROUGH-HOLES divided into smaller groups of 8x8 = SUBARRAYS
- 48 SUBARRAYS



What is an OpenArray® plate?

- Steel plate containing 4 x 12 subarrays comprised of 8 x 8 through-holes for a total of 3072 through-holes.
- Each through-hole hosts a single RT-PCR reaction
- Each through-hole can be compared to a well in a conventional plate



MicroRNA QuantStudio™ 12K Flex Panels

- Broad coverage
 - Run three samples per OpenArray® plate
 - 818 assays per sample (758 unique assays)
 - > 3 positive and 1 negative/process control per subarray
- High-throughput
 - 12 samples per ~2.5 hr instrument run
 - 48 samples per 8hr working day
- Cost effective
 - Rapid sample screening with minimal hands-on time
- Minimal sample requirement
 - 100ng of total RNA recommended for a full profile



OpenArray® miRNA Profiling Panels

OpenArray® Human MicroRNA Panel QuantStudio™ 12K Flex

- Minimum order size 1 panel
- Inventoried
- 2-3 days shipping
- P/N 4470187

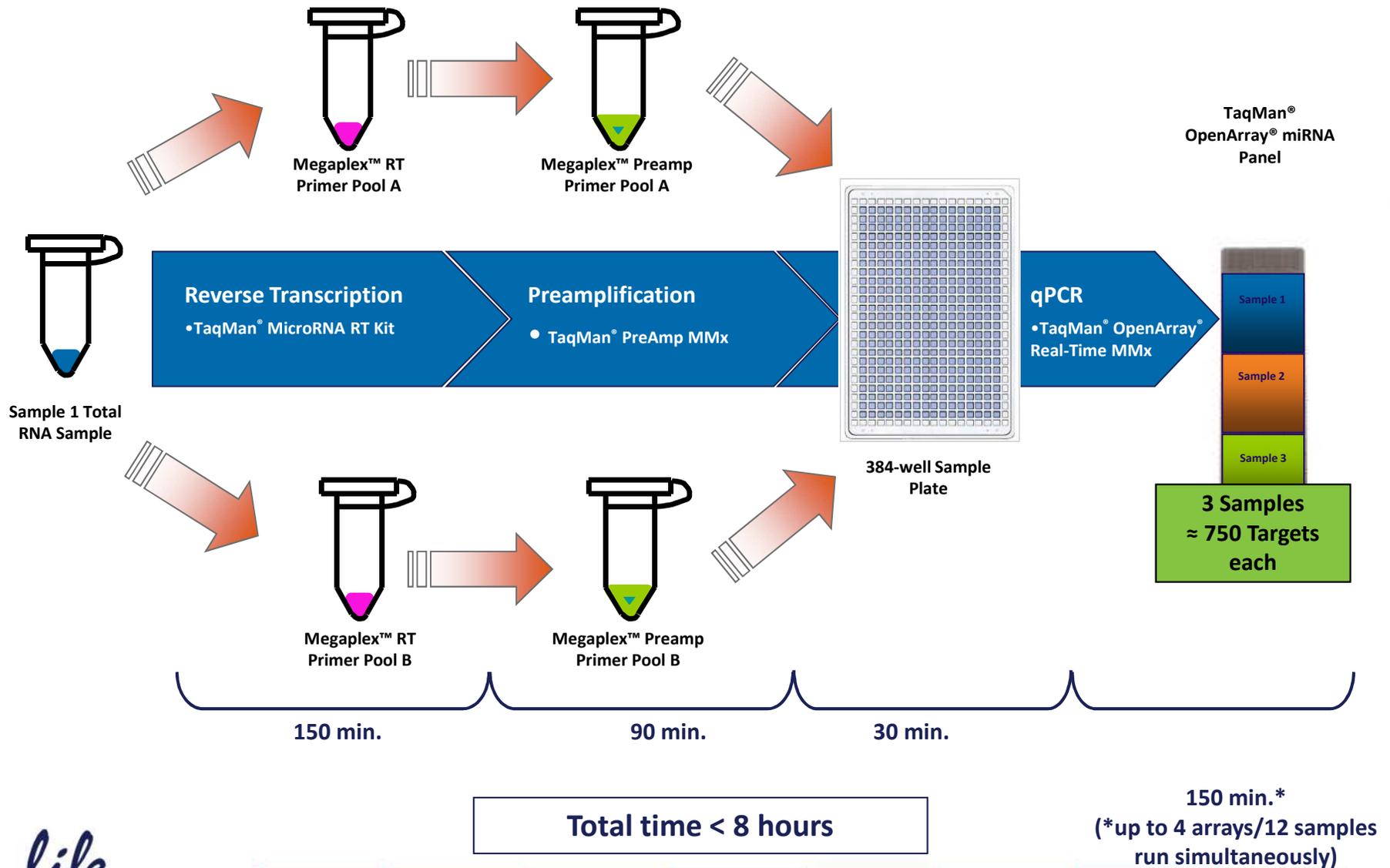


OpenArray® Rodent MicroRNA Panel QuantStudio™ 12K Flex

- Minimum order size 1 panel
- Inventoried
- 2-3 days shipping
- P/N 4470188



OpenArray[®] miRNA Profiling Workflow

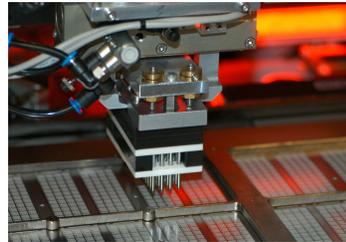


OpenArray® Block User Workflow Overview

At Life Technologies



TaqMan® assays ordered on-line



The assays are spotted on the OpenArray® plate



OpenArray® plates staked in case

At the Researcher's Lab



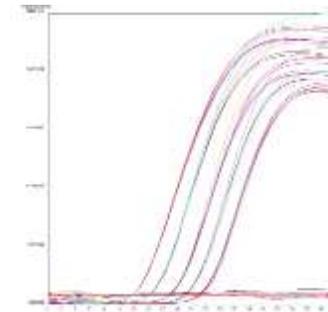
Load your samples with Master Mix onto the OpenArray® plate



Place lid on to array case, load on to carrier



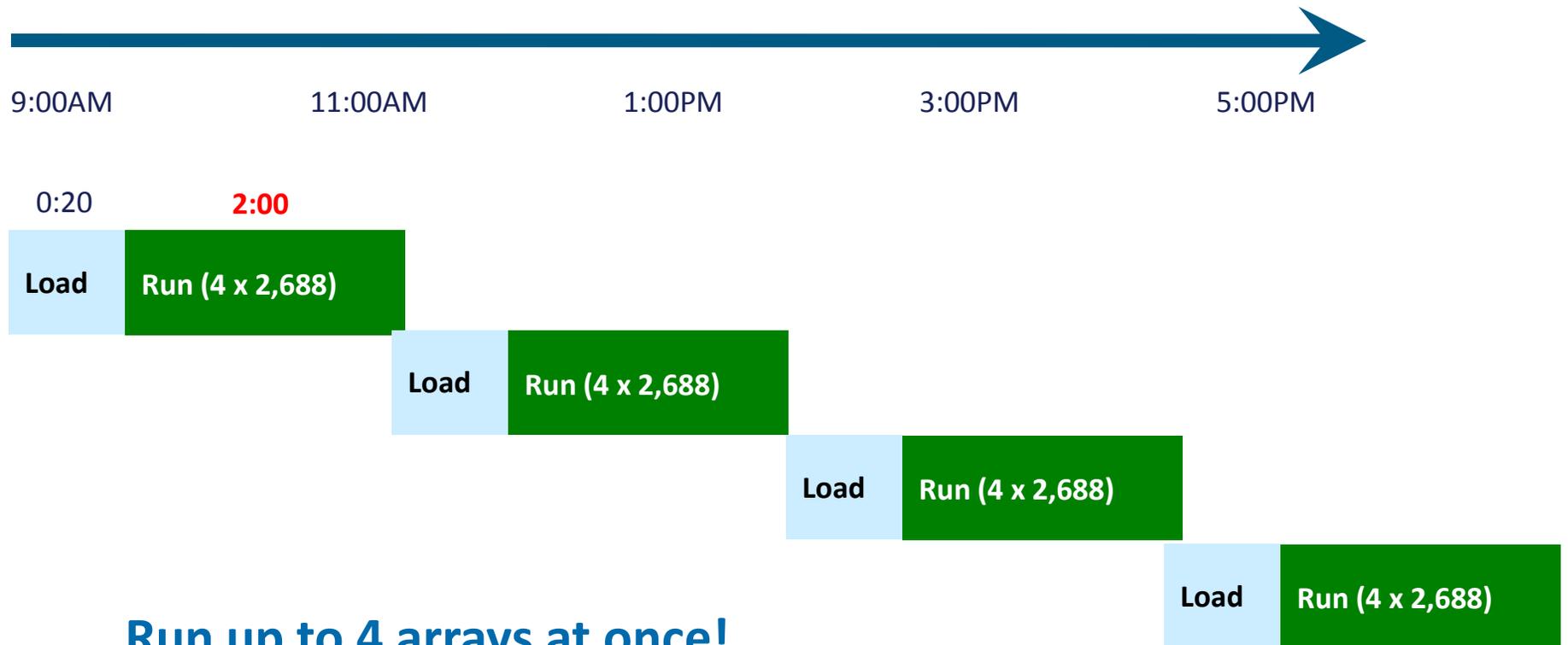
Cycle and image up to 4 OpenArray® plates



Results!



Gene Expression/miRNA on QuantStudio™ 12K Flex OpenArray® Block

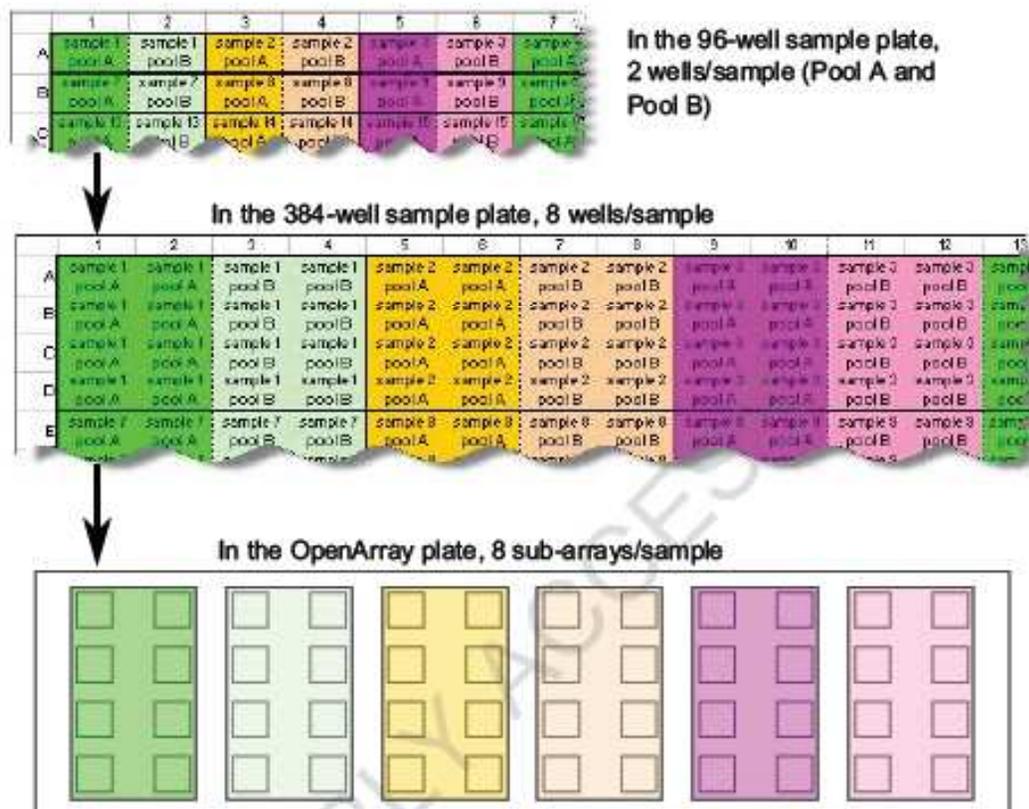


Run up to 4 arrays at once!
16 arrays/ day
>43,000 reactions, 1 operator



Sample Prep

The starting material is total RNA or cell lysate. 50 to 200 ng of input total RNA. For most tissues, 100 ng of total RNA produces a comprehensive microRNA profile with preamplification.



RT and pre-amp

OpenArray® sample+PCR Master mix

OpenArray® loading, Pool A and pool B



Step 1 . RT

Prepare the RT Reaction Mix

1. Thaw the following on ice:

- Megaplex™ RT Primers
- TaqMan® MicroRNA Reverse Transcription Kit components
- MgCl₂ (supplied with the Megaplex™ RT Primers)

2. Combine the following in each of two 1.5-mL microcentrifuge tubes (one for Pool A, the other for Pool B) + 3 uL of total RNA (100 ng)

RT Reaction Mix Components	Volume per reaction	Volume for 3 reactions ¹
Megaplex™ RT Primers (10X), Pool A	0.75 µL	2.5 µL
dNTPs with dTTP (100 mM)	0.15 µL	0.5 µL
MultiScribe™ Reverse Transcriptase (50 U/µL)	1.50 µL	5.1 µL
10X RT Buffer	0.75 µL	2.5 µL
MgCl ₂ (25 mM)	0.90 µL	3.0 µL
RNase Inhibitor (20 U/µL)	0.09 µL	0.3 µL
Nuclease-free water	0.35 µL	1.2 µL
Total	4.50 µL	15.1 µL

¹ Includes 12.5% excess for loss from pipetting.

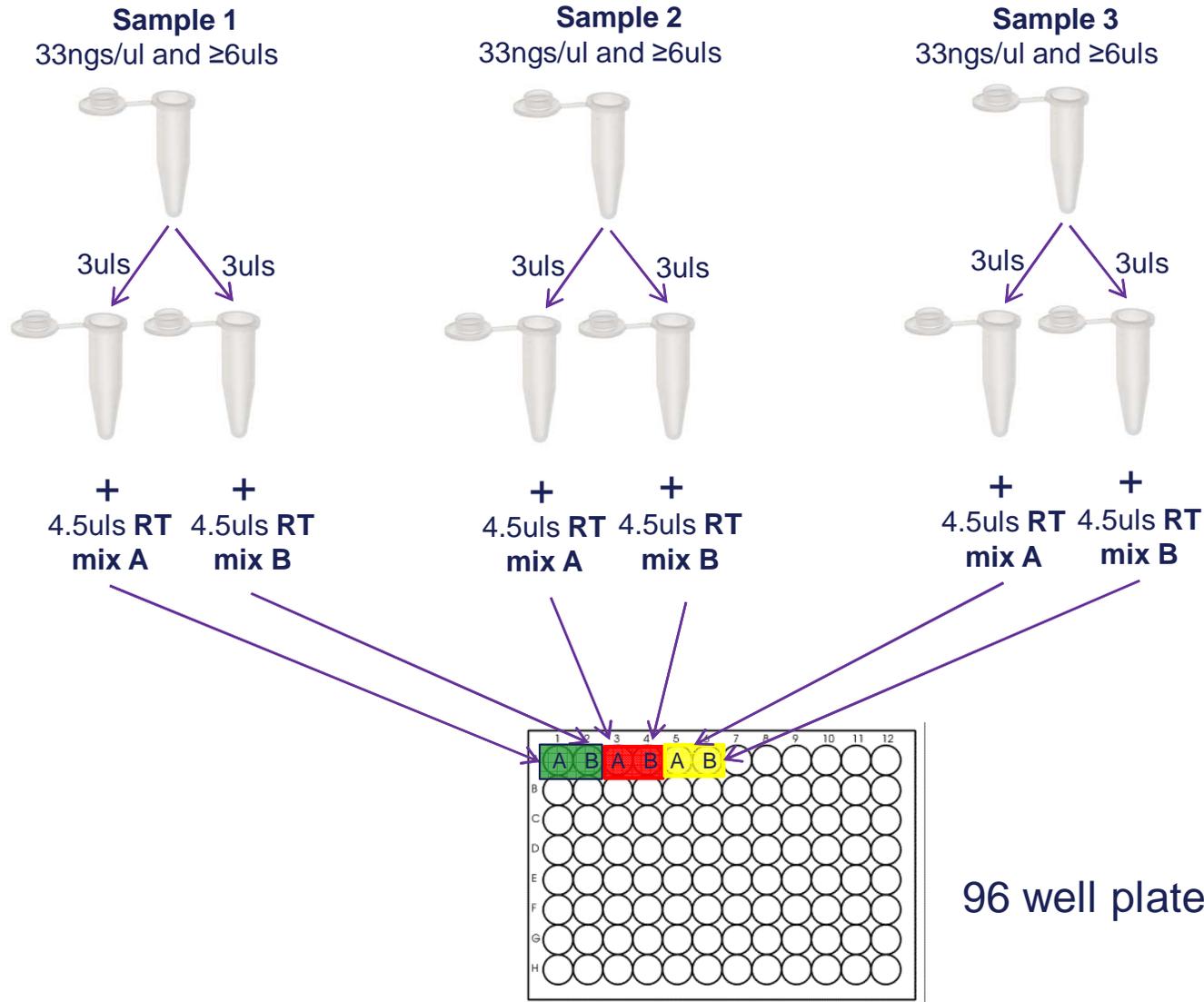
Stage	Temp	Time
Cycle (40 cycles)	16°C	2 min
	42°C	1 min
	50°C	1 sec
Hold	85°C	5 min
Hold	4°C	∞

2. Load, then run the plate.

STOPPING POINT If needed, you can store the RT product (cDNA) at -15 to -25°C for up to 1 month.



Step 1 : Megaplex™ RT reaction: 96 well plate



RT reaction ~2.5h



2. Pre-amplification with Megaplex™ pools A and B

The preamplification reaction has a final volume of 25 µL and contains:

- 2.5 µL RT product
- 22.5 µL PreAmp reaction mix, pool A in one tube and pool B in another

PreAmp Reaction Mix components	Volume for 1 reaction	Volume for 3 reactions ²
2X TaqMan® PreAmp Master Mix	12.5 µL	42.4 µL
Megaplex™ PreAmp Primers (10X), Pool A or Pool B ¹	2.5 µL	8.4 µL
Nuclease-free water	7.5 µL	25.3 µL
Total	22.5 µL	76.1 µL

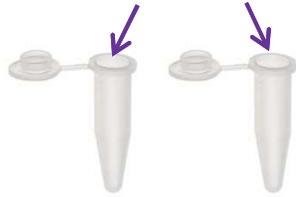
1 Use Pool A in one tube, and Pool B in the other.

2 Includes 12.5% excess for volume loss from pipetting.

Stage	Temp	Time
Hold	95°C	10 min
Hold	55°C	2 min
Hold	72°C	2 min
Cycle (12 cycles)	95°C	15 sec
	60°C	4 min
Hold ¹	99.9°C	10 min
Hold	4°C	∞

Step 2 : Pre-amplification

For sample 1
prepare pre-amp
mmix A and B



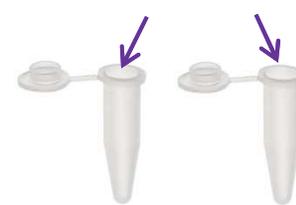
22.5 uls 22.5 uls

For sample 2
prepare pre-amp
mmix A and B



22.5 uls 22.5 uls

For sample 3
prepare pre-amp
mmix A and B



22.5 uls 22.5 uls

add

2.5 uls RT
reaction A
from Step 1

2.5 uls RT
reaction B
from Step 1

add

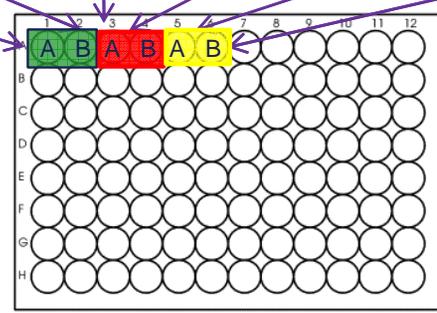
2.5 uls RT
reaction A
from Step 1

2.5 uls RT
reaction B
from Step 1

add

2.5 uls RT
reaction A
from Step 1

2.5 uls RT
reaction B
from Step 1

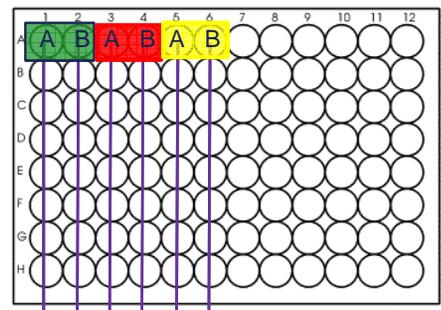


96 well plate (2nd)

Pre-amp reaction ~1.5h



Step 3 :Dilute the Pre-amplification Product



96 well plate from previous step

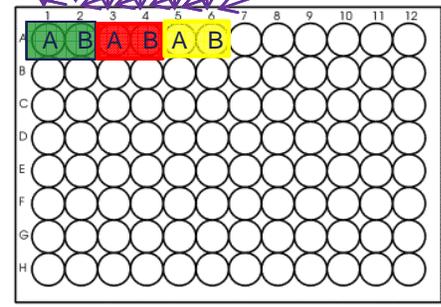
4 μ L

+

156 μ L

Preamplified cDNA

0.1x TE pH 8.0

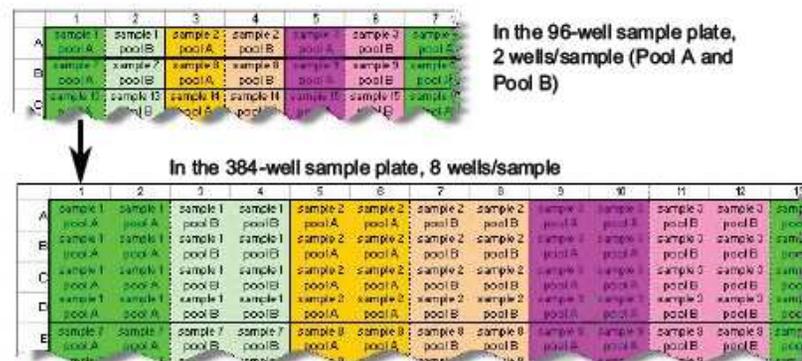


96 well plate (3rd)

4. Prepare OpenArray[®] sample plate

To prepare OpenArray[®] sample plate you need:

- PCR master mix in 1.5 mL tube
- aliquots of diluted pre-amp pool A and pool B products



1. Aliquot 22.5 μ L of TaqMan OpenArray[®] reaction mix into each of the 2 wells per sample of a clean 96-well plate.
2. For each sample, add 22.5 μ L of diluted Pool A PreAmp and 22.5 μ L of diluted Pool B PreAmp into the other well.
3. Transfer 5 μ L of PCR mix in sample plate.

Step 4: prepare PCR reaction

Prepare 22.5uls of PCR Mmix for Sample 1A and 1B



Prepare 22.5uls of PCR Mmix for Sample 2A and 2B



Prepare 22.5uls of PCR Mmix for Sample 3A and 3B



add

22.5 uls of diluted pre-amplified sample from step 3

22.5 uls of diluted pre-amplified sample from step 3

add

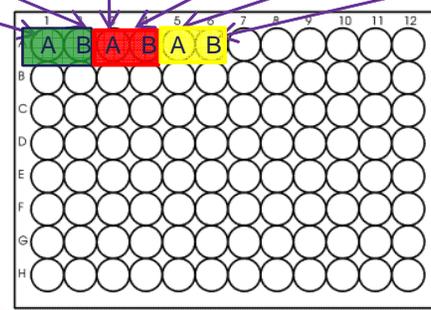
22.5 uls of diluted pre-amplified sample from step 3

22.5 uls of diluted pre-amplified sample from step 3

add

22.5 uls of diluted pre-amplified sample from step 3

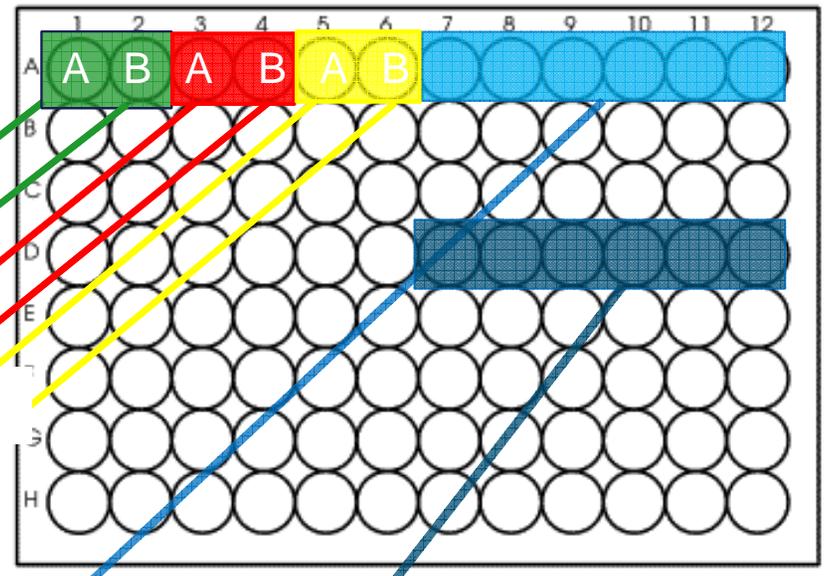
22.5 uls of diluted pre-amplified sample from step 3



96 well plate (4th)

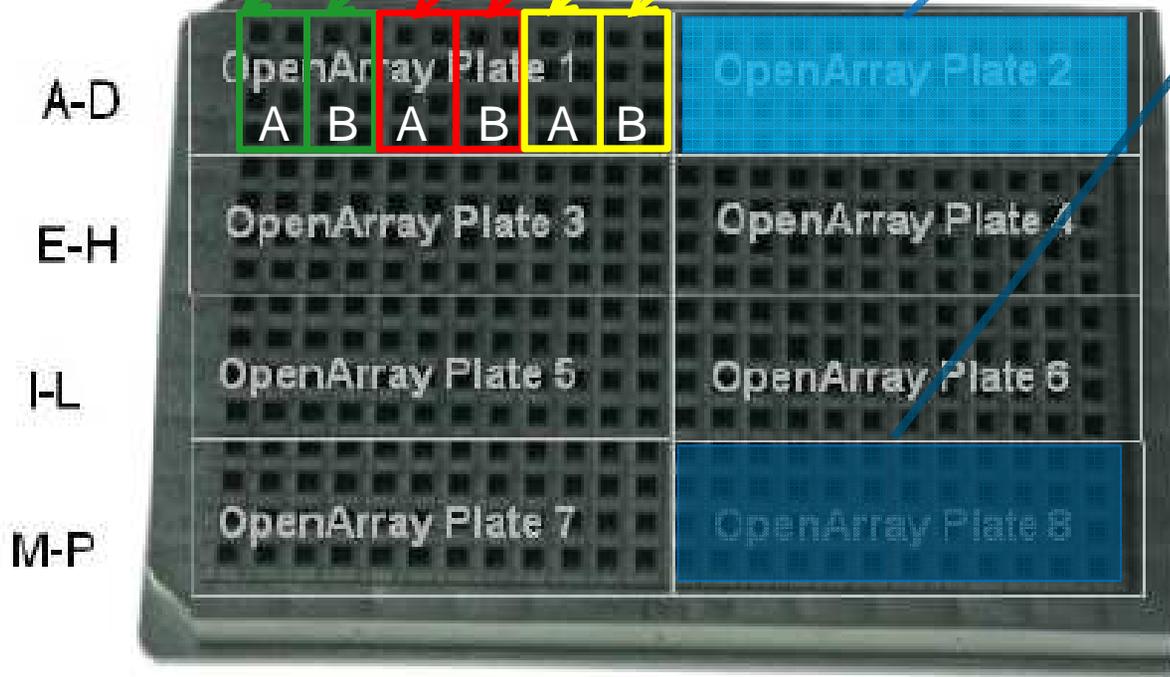


96 well plate from previous step



5uls in each well

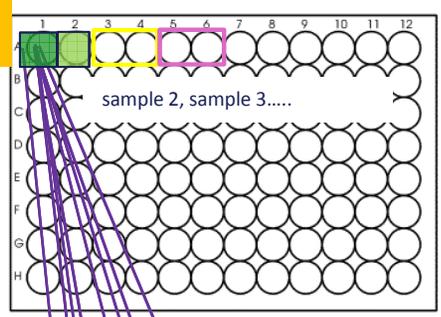
1-12



You MUST use a TaqMan®
OpenArray® 384-Well
Sample Plate



45ul in each well

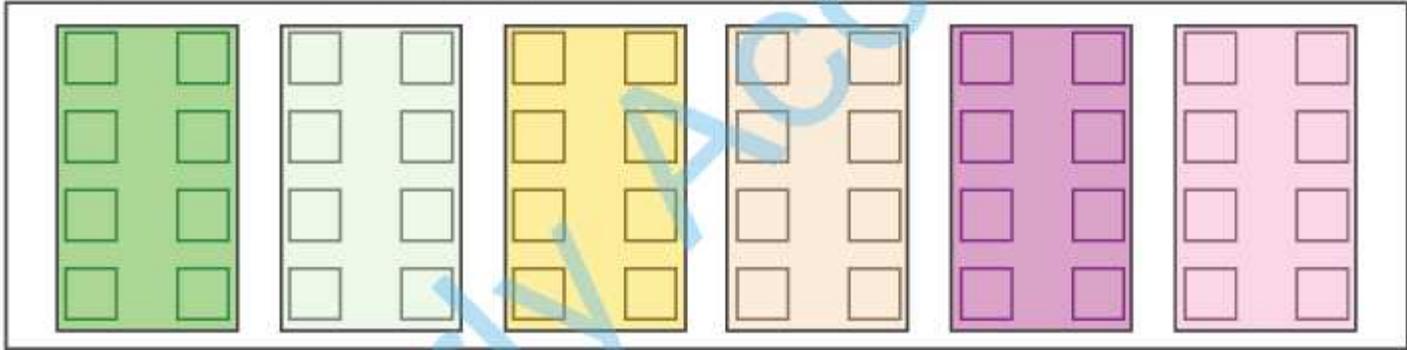


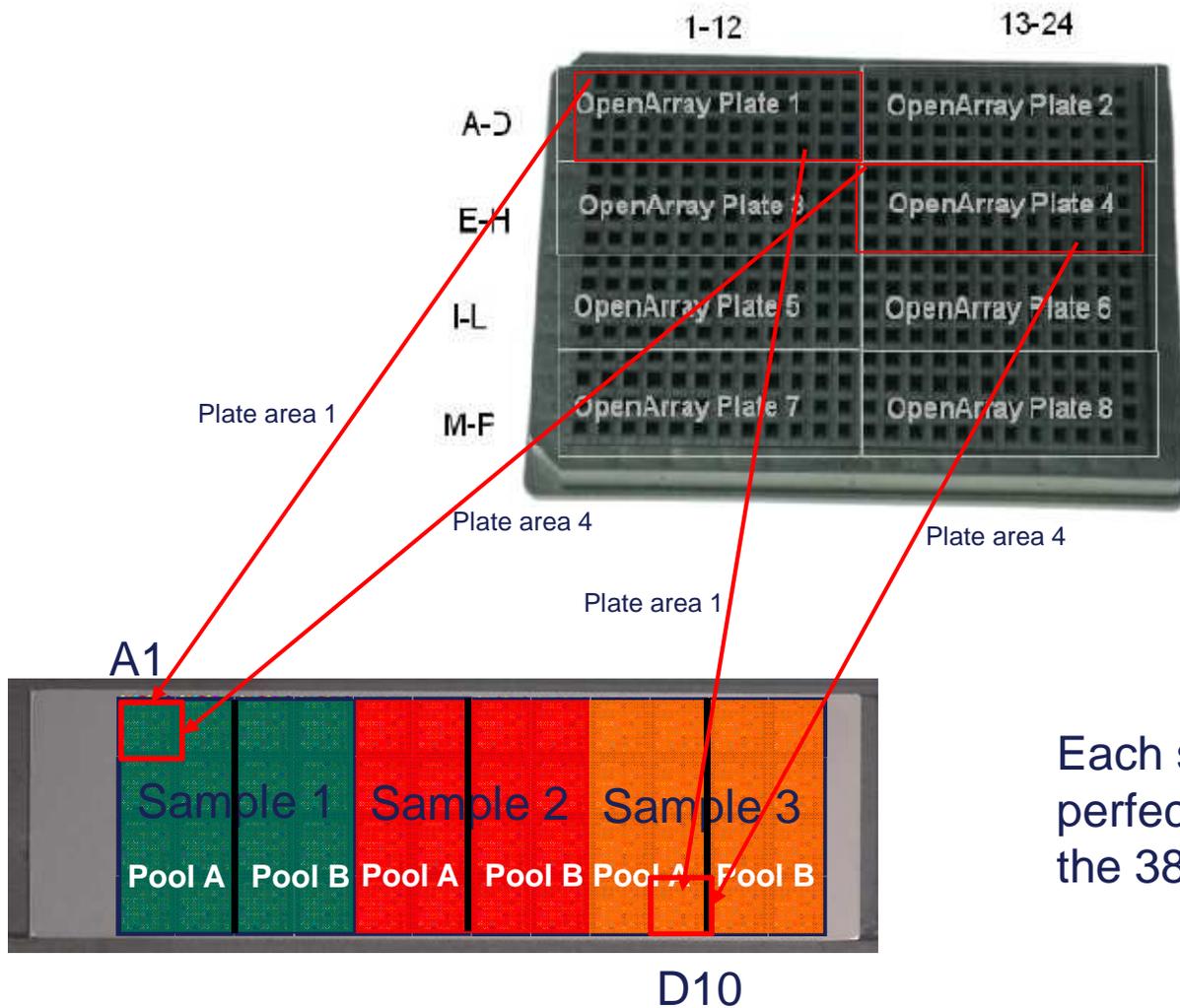
TaqMan® OpenArray® 384-Well Sample plate

5ul in each well

	1	2	3	4	5	6	7	8	9	10	11	12	13
A	sample 1 pool A	sample 1 pool A	sample 1 pool B	sample 1 pool B	sample 2 pool A	sample 2 pool A	sample 2 pool B	sample 2 pool B	sample 3 pool A	sample 3 pool A	sample 3 pool B	sample 3 pool B	sample 3 pool B
B	sample 1 pool A	sample 1 pool A	sample 1 pool B	sample 1 pool B	sample 2 pool A	sample 2 pool A	sample 2 pool B	sample 2 pool B	sample 3 pool A	sample 3 pool A	sample 3 pool B	sample 3 pool B	sample 3 pool B
C	sample 1 pool A	sample 1 pool A	sample 1 pool B	sample 1 pool B	sample 2 pool A	sample 2 pool A	sample 2 pool B	sample 2 pool B	sample 3 pool A	sample 3 pool A	sample 3 pool B	sample 3 pool B	sample 3 pool B
D	sample 1 pool A	sample 1 pool A	sample 1 pool B	sample 1 pool B	sample 2 pool A	sample 2 pool A	sample 2 pool B	sample 2 pool B	sample 3 pool A	sample 3 pool A	sample 3 pool B	sample 3 pool B	sample 3 pool B
E	sample 7 pool A	sample 7 pool A	sample 7 pool B	sample 7 pool B	sample 8 pool A	sample 8 pool A	sample 8 pool B	sample 8 pool B	sample 9 pool A	sample 9 pool A	sample 9 pool B	sample 9 pool B	sample 9 pool B

In the OpenArray plate, 8 sub-arrays/sample



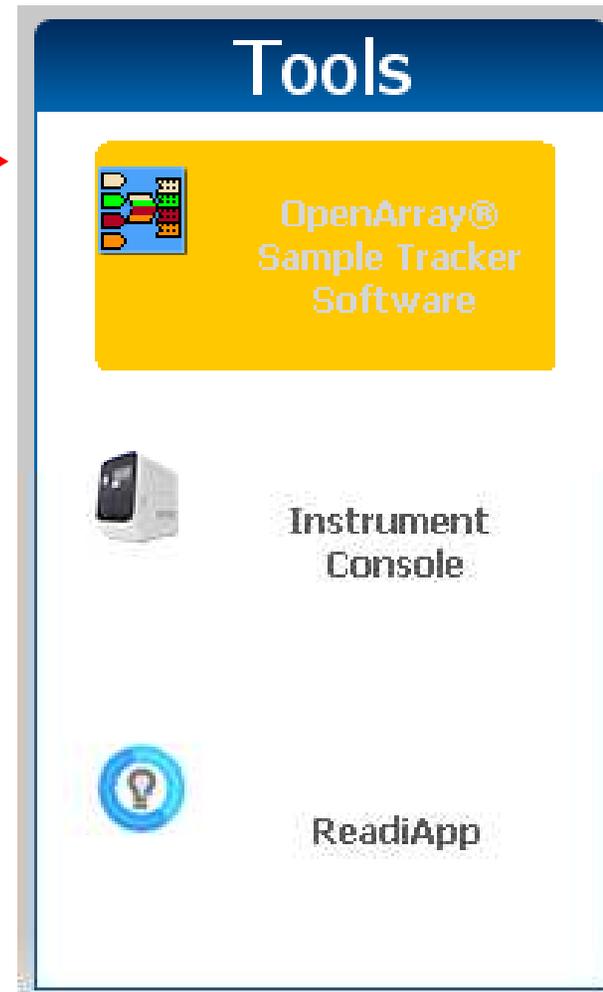


Each subarray overlaps perfectly with a single well in the 384 well sample plate

SampleTracker

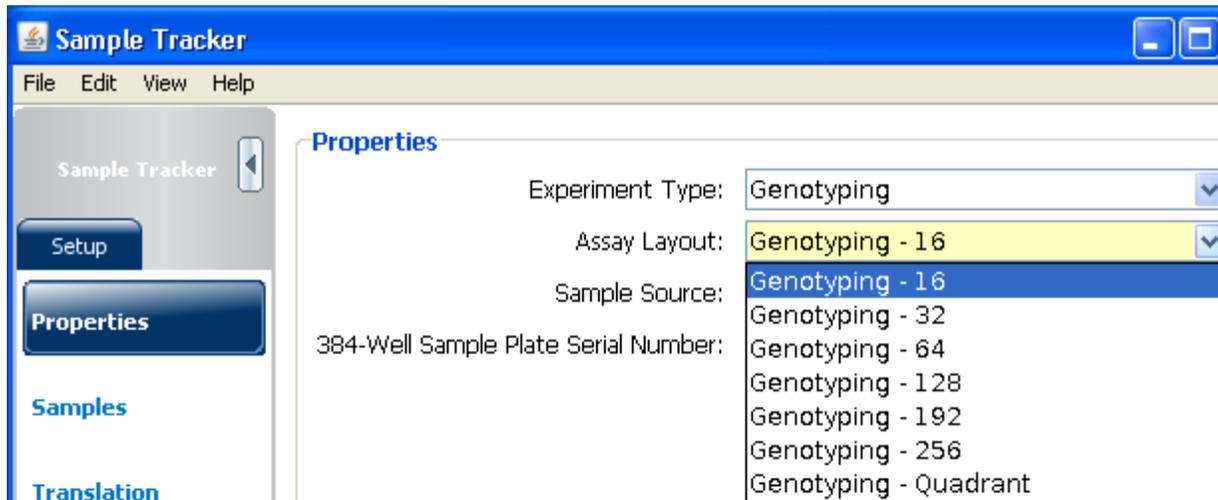
Sample Tracker

- New utility to map 96-well plates with DNA onto 384-well sample plates or OpenArray® plates
- Launch from instrument home screen
- Also a standalone application
- Creates 384-well DNA sample plate and OpenArray® plate .csv files for all assay formats of genotyping and gene expression OpenArray® plates
- For fixed spacing 12 tip multichannel pipettes
- Exports plate maps in .pdf or Excel® format.

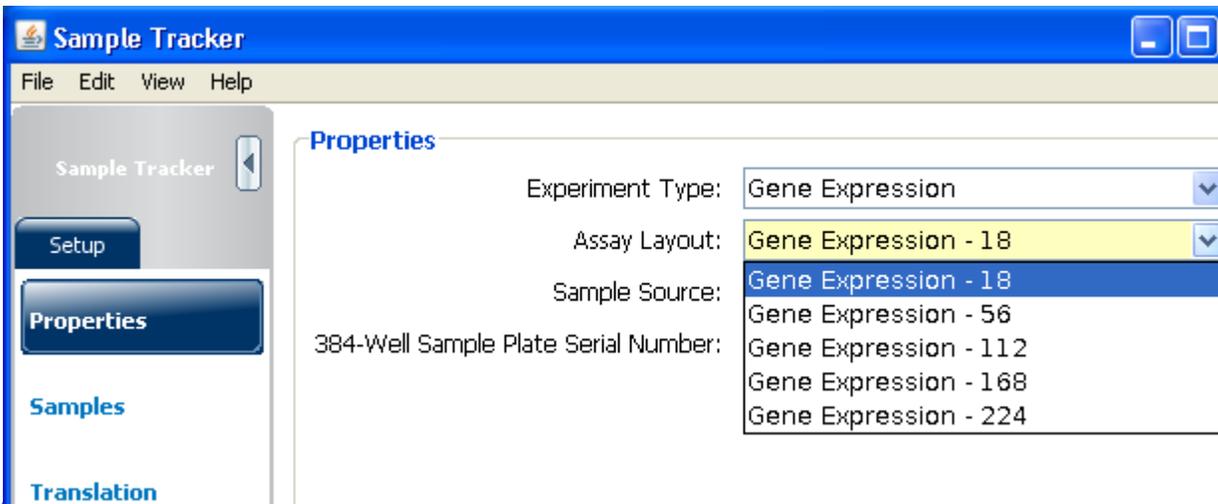


Sample Tracker – Properties

- Under Properties menu - select experiment type and assay layout



Genotyping



Gene Expression

Sample Tracker - Samples

- Under Samples menu - import 96-well plate .csv files

The screenshot shows the 'Sample Tracker' application interface. On the left is a sidebar with 'Setup', 'Properties', 'Samples', and 'Translation' options. The main area displays 'Plate 1' with a 'View Table' button and zoom controls. A 12x4 grid shows sample IDs: Row A (S101-S112), Row B (S113-S124), Row C (S125-S136), and Row D (S137-S148). 'Import' and 'Clear' buttons are on the right.

- Input .csv file format for 96-well plates:

- Files are created by user

SamplePlate. SerialNumber	SampleInfo. Address	SampleInfo. SampleID	SampleInfo. Description	SampleInfo. Dilution	SamplePlateSelectedArea. SelectedAreaIndex
1	A01	S101			
1	A02	S102			
1	A03	S103			

Sample Tracker - Translation

- The Translation menu shows mapping of 96-well plates into the 384-well sample plate when the “384 Plate” tab is selected.
- The 384-well sample plate .csv file is exported from here for upload to AccuFill™ System (Sample Integration)

Sample Tracker v1.0

File Edit Instrument Analysis Tools Help

New Experiment Open Save Close Import Create Slide Print Report

Sample Tracker

File Edit View Help

384 Plate OpenArray

Show Swap **Export** Print PDF Export Excel

Sample/Intermediary Plates

View Table Zoom In Zoom Out Fit to Size

	1	2	3	4	5	6	7	8	9	10	11	12
A	S101	S102	S103	S104	S105	S106	S107	S108	S109	S110	S111	S112
B	S113	S114	S115	S116	S117	S118	S119	S120	S121	S122	S123	S124
C	S125	S126	S127	S128	S129	S130	S131	S132	S133	S134	S135	S136
D	S137	S138	S139	S140	S141	S142	S143	S144	S145	S146	S147	S148
E	S149	S150	S151	S152	S153	S154	S155	S156	S157	S158	S159	S160
F	S161	S162	S163	S164	S165	S166	S167	S168	S169	S170	S171	S172
G	S173	S174	S175	S176	S177	S178	S179	S180	S181	S182	S183	S184
H	S185	S186	S187	S188	S189	S190	S191	S192	S193	S194	S195	S196

View Table Zoom In Zoom Out Fit to Size

	1	2	3	4	5	6	7	8	9	10	11	12
A	S301	S302	S303	S304	S305	S306	S307	S308	S309	S310	S311	S312
B	S313	S314	S315	S316	S317	S318	S319	S320	S321	S322	S323	S324
C	S325	S326	S327	S328	S329	S330	S331	S332	S333	S334	S335	S336
D	S337	S338	S339	S340	S341	S342	S343	S344	S345	S346	S347	S348
E	S349	S350	S351	S352	S353	S354	S355	S356	S357	S358	S359	S360
F	S361	S362	S363	S364	S365	S366	S367	S368	S369	S370	S371	S372
G	S373	S374	S375	S376	S377	S378	S379	S380	S381	S382	S383	S384
H	S385	S386	S387	S388	S389	S390	S391	S392	S393	S394	S395	S396

View Table Zoom In Zoom Out Fit to Size

	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	S101	S113	S102	S114	S103	S115	S104	S116	S105	S117	S106	S118	S107	S119	S108	S120	S109	S121	S110	S122	S111	S123	S112	S124
B	S125	S137	S126	S138	S127	S139	S128	S140	S129	S141	S130	S142	S131	S143	S132	S144	S133	S145	S134	S146	S135	S147	S136	S148
C	S149	S161	S150	S162	S151	S163	S152	S164	S153	S165	S154	S166	S155	S167	S156	S168	S157	S169	S158	S170	S159	S171	S160	S172
D	S173	S185	S174	S186	S175	S187	S176	S188	S177	S189	S178	S190	S179	S191	S180	S192	S181	S193	S182	S194	S183	S195	S184	S196
E	S201	S213	S202	S214	S203	S215	S204	S216	S205	S217	S206	S218	S207	S219	S208	S220	S209	S221	S210	S222	S211	S223	S212	S224
F	S225	S237	S226	S238	S227	S239	S228	S240	S229	S241	S230	S242	S231	S243	S232	S244	S233	S245	S234	S246	S235	S247	S236	S248
G	S249	S261	S250	S262	S251	S263	S252	S264	S253	S265	S254	S266	S255	S267	S256	S268	S257	S269	S258	S270	S259	S271	S260	S272
H	S273	S285	S274	S286	S275	S287	S276	S288	S277	S289	S278	S290	S279	S291	S280	S292	S281	S293	S282	S294	S283	S295	S284	S296
I	S301	S313	S302	S314	S303	S315	S304	S316	S305	S317	S306	S318	S307	S319	S308	S320	S309	S321	S310	S322	S311	S323	S312	S324



Sample Tracker - Translation

- The Translation menu shows mapping of 96-well plates into the 384-well sample plate when the “384 Plate” tab is selected.
- The 384-well.csv file can be exported and placed in: C:\OpenArray\OpenArray Plates for integration with TPF/SPF file in AccuFill™ System software.

The screenshot displays the Sample Tracker v1.0 software interface. The main window shows a grid of sample data under the '384 Plate' tab. A dialog box titled 'Select Plates to Export' is open, listing several plate options. The '384 Plate' option is selected with a checkmark. The 'Export Directory' field is set to 'penArray\OpenArray Plates' with a 'Browse...' button next to it. The 'Export' button in the background is circled in red.

	1	2	3	4	5	6	7	8	9	10
A	S101	S102	S103	S104	S105	S106	S107	S108	S109	S110
B	S113	S114	S115	S116	S117	S118	S119	S120	S121	S122
C	S125	S126	S127	S128	S129	S130	S131	S132	S133	S134
D	S137	S138	S139	S140	S141	S142	S143	S144	S145	S146
E	S149	S150	S151	S152	S153	S154	S155	S156	S157	S158
F	S161	S162	S163	S164	S165	S166	S167	S168	S169	S170
G	S173	S174	S175	S176	S177	S178	S179	S180	S181	S182
H	S185	S186	S187	S188	S189	S190	S191	S192	S193	S194

	1	2	3	4	5	6	7	8	9	10
A	S301	S302	S303	S304	S305	S306	S307	S308	S309	S310
B	S313	S314	S315	S316	S317	S318	S319	S320	S321	S322
C	S325	S326	S327	S328	S329	S330	S331	S332	S333	S334
D	S337	S338	S339	S340	S341	S342	S343	S344	S345	S346
E	S349	S350	S351	S352	S353	S354	S355	S356	S357	S358
F	S361	S362	S363	S364	S365	S366	S367	S368	S369	S370
G	S373	S374	S375	S376	S377	S378	S379	S380	S381	S382
H	S385	S386	S387	S388	S389	S390	S391	S392	S393	S394

	1	2	3	4	5	6	7	8	9	10
A	S101	S113	S102	S114	S103	S115	S104	S116	S105	S117
B	S125	S137	S126	S138	S127	S139	S128	S140	S129	S141
C	S149	S161	S150	S162	S151	S163	S152	S164	S153	S165
D	S173	S185	S174	S186	S175	S187	S176	S188	S177	S189
E	S201	S213	S202	S214	S203	S215	S204	S216	S205	S217
F	S225	S237	S226	S238	S227	S239	S228	S240	S229	S241
G	S249	S261	S250	S262	S251	S263	S252	S264	S253	S265
H	S273	S285	S274	S286	S275	S287	S276	S288	S277	S289
T	S301	S313	S302	S314	S303	S315	S304	S316	S305	S317

Sample Tracker - Translation

- The OpenArray tab shows a map of how the color-coded 96-well plates are loaded on the OpenArray plates.

Sample Tracker

File Edit View Help

Sample Tracker

384 Plate OpenArray

Setup

Properties

Samples

Translation

Zoom In Zoom Out Fit to Size

Zoom In Zoom Out Fit to Size

Zoom in to see sample names

OpenArray 1

OpenArray 2

	1	2	3	4	5	6	7	8	9	10	11	12
a												
b												
c												
d												
A												
e												
f												
g												
h												
A												
b												
c												
d												
E												
f												
g												
h												
C												
e												
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h												
D												
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g												
h												

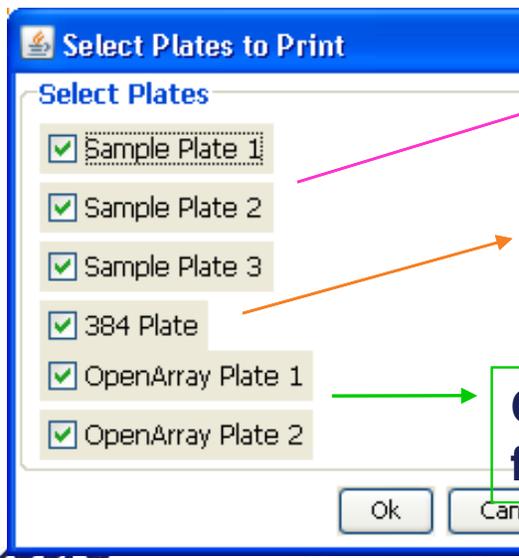
	1	2	3	4	5	6	7	8	9	10	11	12
a												
b												
c												
d												
A												
e												
f												
g												
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g												
h												
D												
e												
f												
g												
h												

Sample Tracker – Translation Features

- The 96-well plates can be swapped.



- The plate maps printed using PDF
- Plate maps can be exported to AccuFill™ System or Data collection softwares



Normal sample file (96w csv format)

AccuFill™ System Software sample/spf integration (384W format csv)

QuantStudio™ Software sample import (OpenArray® format csv)

Software Workflow Basics



SampleTracker

AccuFill™ Software

QuantStudio™ 12K Flex Software

**Genotyper v1.2
ExpressionSuite v1.0
DigitalSuite**

.CSV

.tpf/.spf

.eds

.las

.edt



File Formats

.CSV



Sample file name

.tpf/.spf



Unique OpenArray® file containing Assay IDs/names
(similar to .txt set up file)

“.tpf” = Genex assays “.spf” = genotyping assays

.eds



Experiment file

.edt



Template file

} Embedded thermal
protocol info

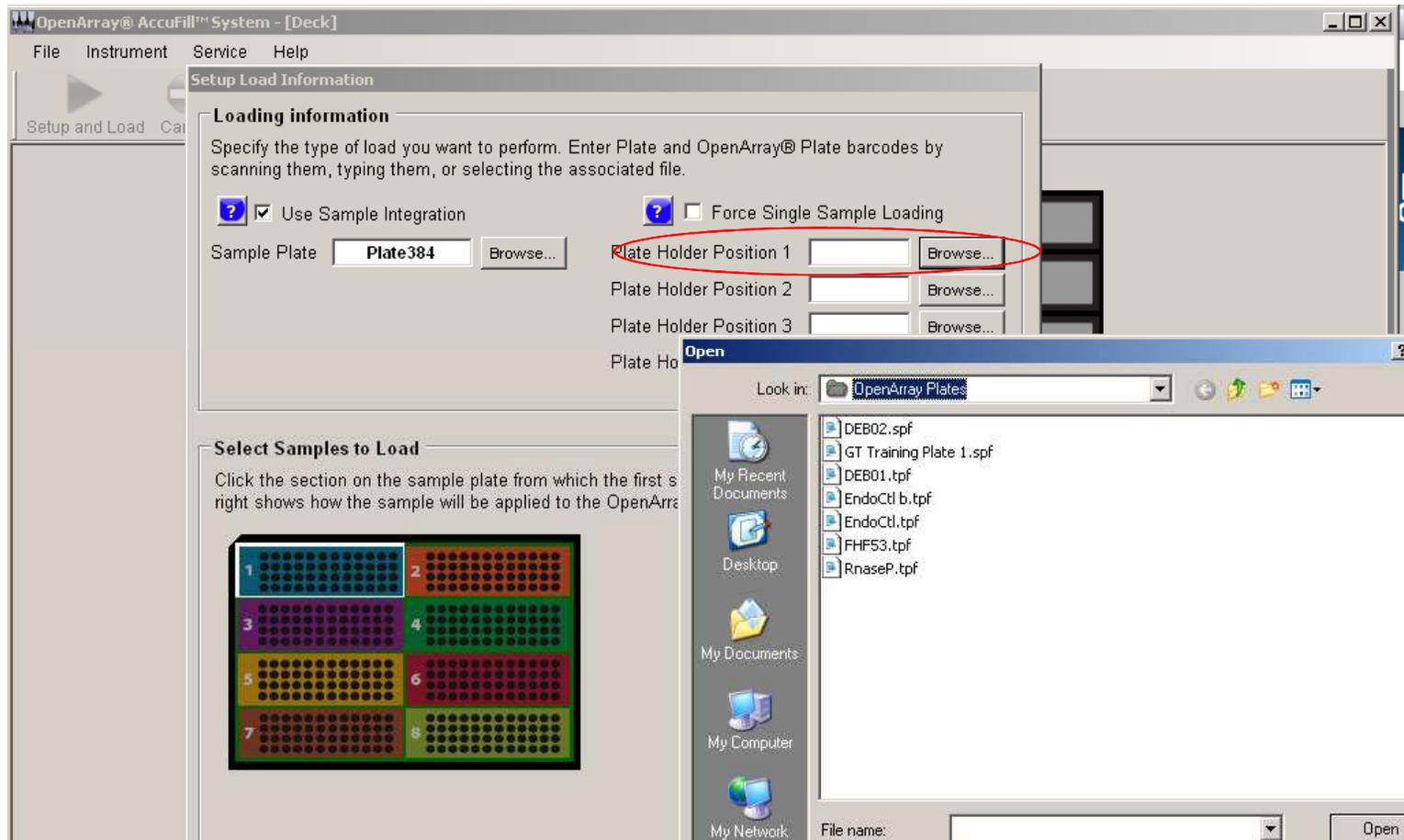
.las



Study file

In AccuFill™ System software, v1.x

- Integrate 384W sample plate + tpf.spf



Starting OpenArray® Run in QuantStudio™ 12K Flex Software

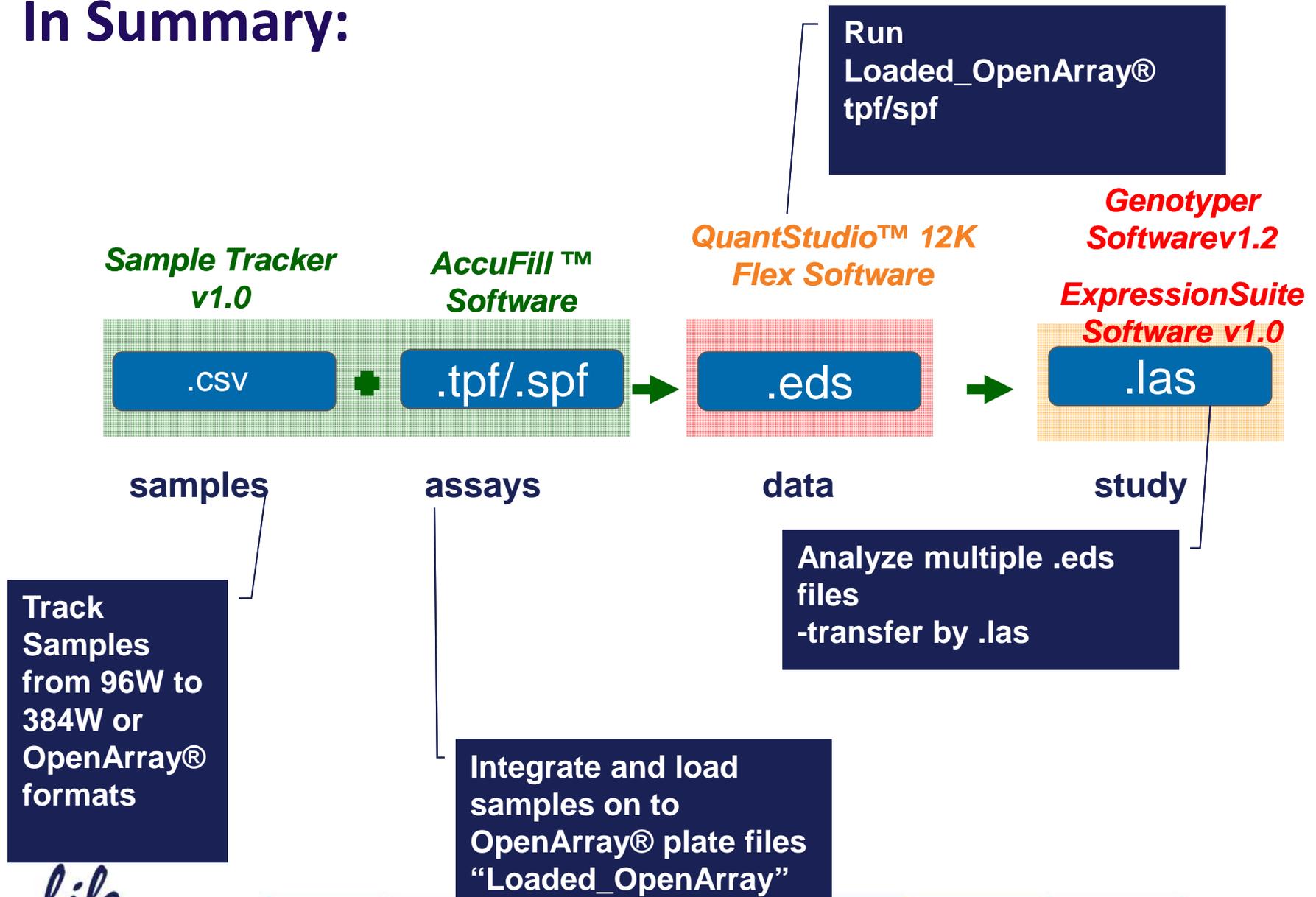
1. From the home screen under the Run menu click **OpenArray®, Get Plate ID**
2. **Browse for file** in Experiment File Location field

The screenshot displays the software interface for starting an OpenArray run. On the left, the 'Run' menu is open, showing the 'OpenArray®' option. The main window is titled 'Setup OpenArray Run' and includes fields for 'OpenArray 1' through '4', 'Run Type' (set to 'Genotyping'), and 'Reagent Type' (set to 'TaqMan'). A 'Browse' button next to the 'Setup File' field for OpenArray 1 is circled in red. Below this, the 'Select Instrument' dialog is open, showing a file explorer view of the 'experiments' folder. The 'Files of type' dropdown is set to 'Setup Files (*.spf;*.tpf)', and the file list shows several files, with one file selected and circled in red. The bottom of the screen features a navigation bar with logos for Invitrogen, Applied Biosystems, Gibco, Molecular Probes, Novex, TaqMan, Ambion, and Ion Torrent.

3. Can start run using **eds, edt, SPF/TPF**



In Summary:



Why are we doing this?

- Samples / sample names get transferred, as follows. We need to be able to track them!



96-well plate: long-term sample storage



384-well plate: used by AccuFill™ System to transfer samples to OpenArray® plate



3072-well OpenArray® plate: for cycling and end-point read



Assign

Show in Table ▼ Select Wells ▼ Group by ▼

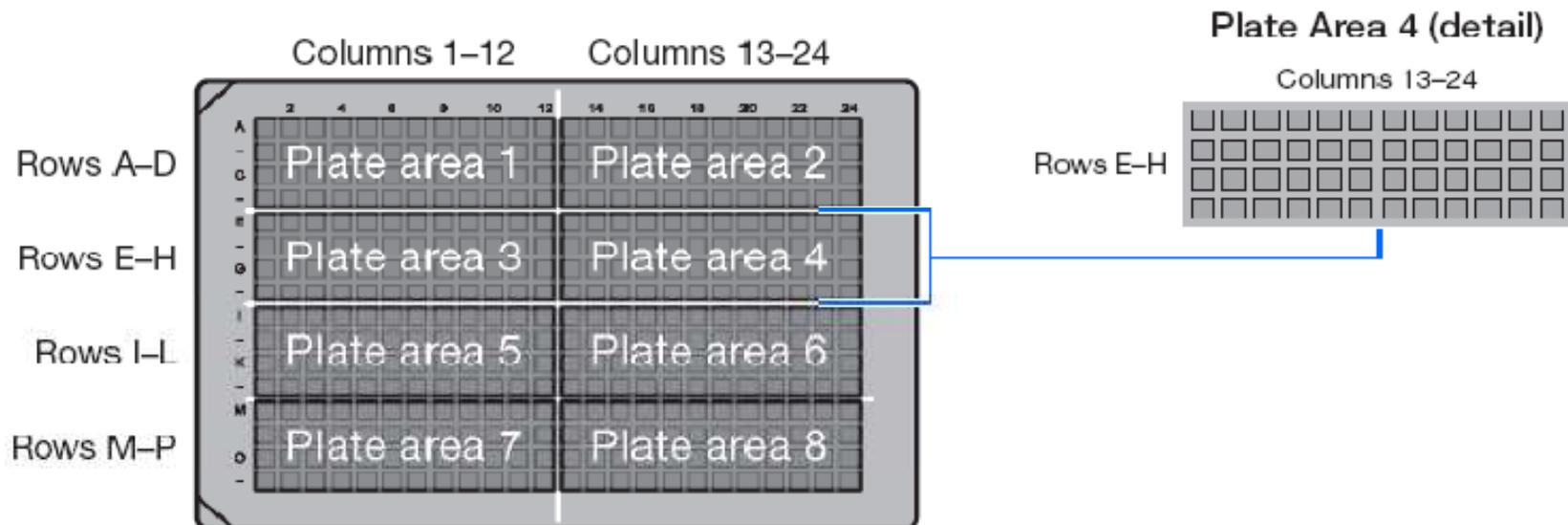
#	Well	Sample	SNP Assay N...	Assay ID	Task
1	A1a1	NA17004	C__177489_10C__177489_10	Unknown	
2	A1a2	NA17004	C__940286_10C__940286_10	Unknown	
3	A1a3	NA17004	C__1046426_10C__1046426_10	Unknown	
4	A1a4	NA17004	C__1085595_10C__1085595_10	Unknown	
5	A1a5	NA17004	C__1213693_10C__1213693_10	Unknown	
6	A1a6	NA17004	C__1240647_1_C__1240647_1	Unknown	
7	A1a7	NA17004	C__1240651_20C__1240651_20	Unknown	
8	A1a8	NA17004	C__1332250_10C__1332250_10	Unknown	
9	A1b1	NA17004	C__1376137_10C__1376137_10	Unknown	
10	A1b2	NA17004	C__1551497_10C__1551497_10	Unknown	
11	A1b3	NA17004	C__1839948_10C__1839948_10	Unknown	
12	A1b4	NA17004	C__1985480_20C__1985480_20	Unknown	
13	A1b5	NA17004	C__2267279_10C__2267279_10	Unknown	
14	A1b6	NA17004	C__2301954_20C__2301954_20	Unknown	
15	A1b7	NA17004	C__2862873_10C__2862873_10	Unknown	
16	A1b8	NA17004	C__3123006_1_C__3123006_1	Unknown	
17	A1c1	NA17004	C__3123485_1_C__3123485_1	Unknown	
18	A1c2	NA17004	C__3168989_10C__3168989_10	Unknown	
19	A1c3	NA17004	C__3197300_10C__3197300_10	Unknown	
20	A1c4	NA17004	C__3237878_10C__3237878_10	Unknown	

QuantStudio™ Software: data analysis



Now it's time to load the Sample Plate

- OpenArray® 384-well SAMPLE plate are used to mix sample with TaqMan® OpenArray® Real-Time PCR Master Mix
 - centrifuge the plate at 1000 rpm, 1 minute
- Using a black Sharpie®, label the sample plate
 - 12 x 4 sections (= area)
 - Each section contains 12-48 individual samples



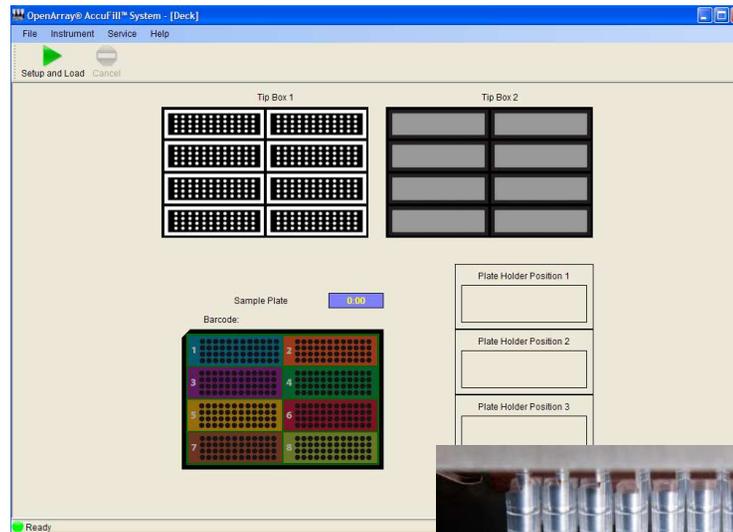
Sample Loading using the OpenArray® AccuFill™ System

Prepare 384-well sample plate

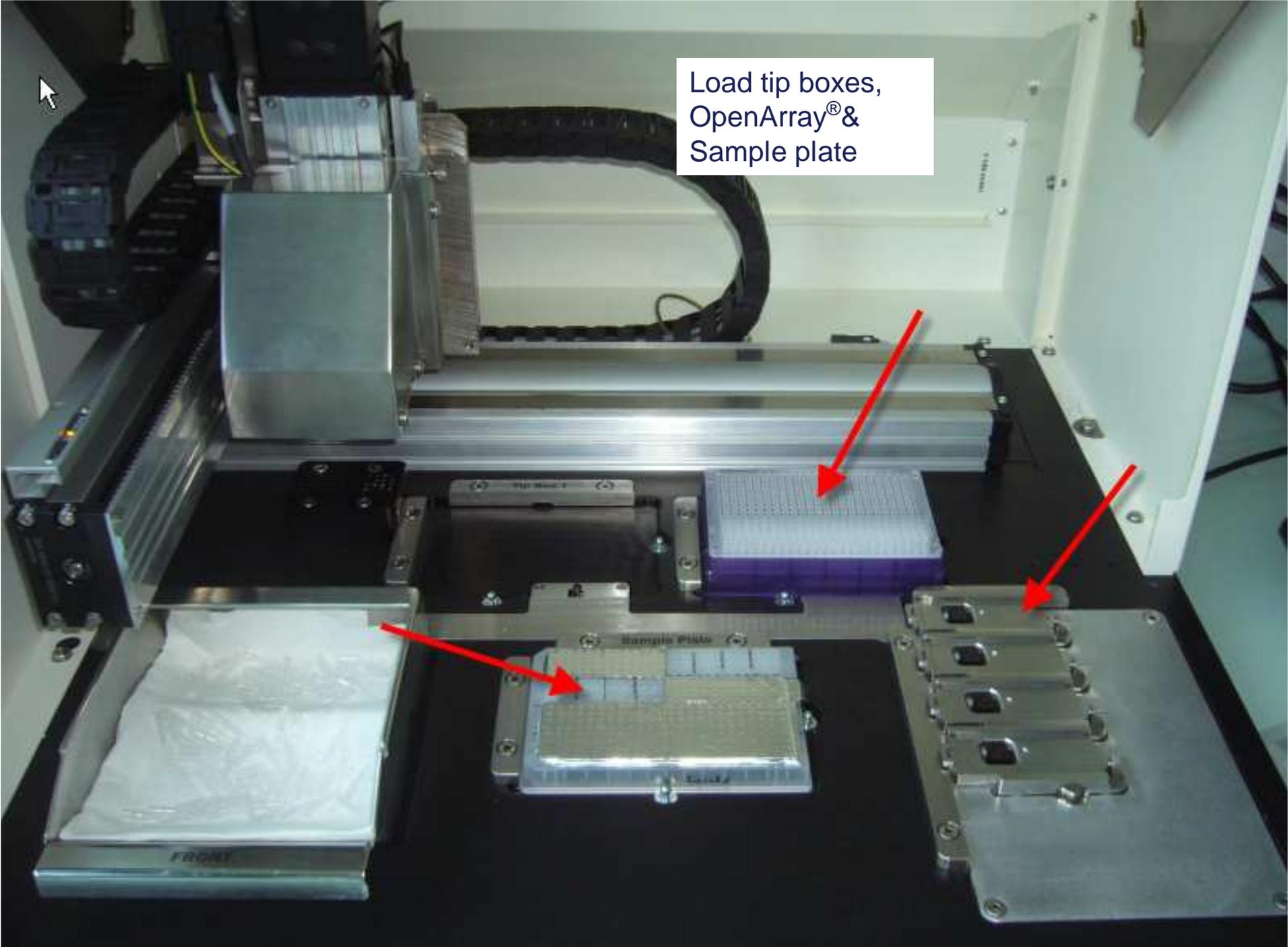
Load tip boxes, OpenArray® & Sample plate

Choose program and press “start”

Remove loaded OpenArray® Plate



Load tip boxes,
OpenArray® &
Sample plate

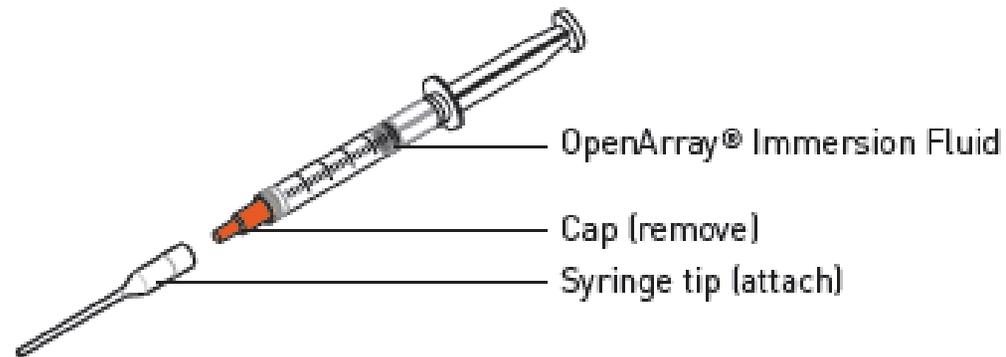


OpenArray[®] plate guidelines

- Thaw plates for **at least 15 minutes** at RT *before* opening the sealed pouches.
- Once you remove them, proceed to The AccuFill™ System.
 - Plates need to be loaded within **one hour** of opening.
- Handle cases with snug, powder-free gloves.
- Only touch OpenArray[®] plates on the case edges.
 - Be careful not to touch the through-holes!
- If you drop an OpenArray[®] plate, discard it.

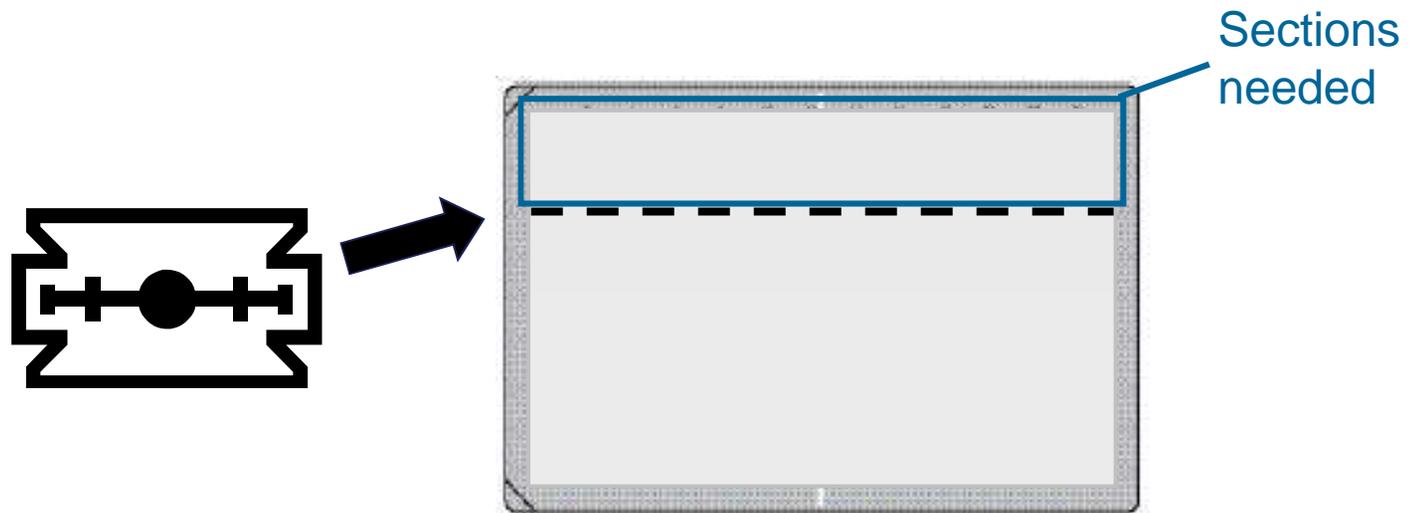
Additional preparations

- While OpenArray® plates are thawing in their pouches, prepare immersion fluid syringes.
 - Simply remove cap, attach the tip, and set on a clean surface.



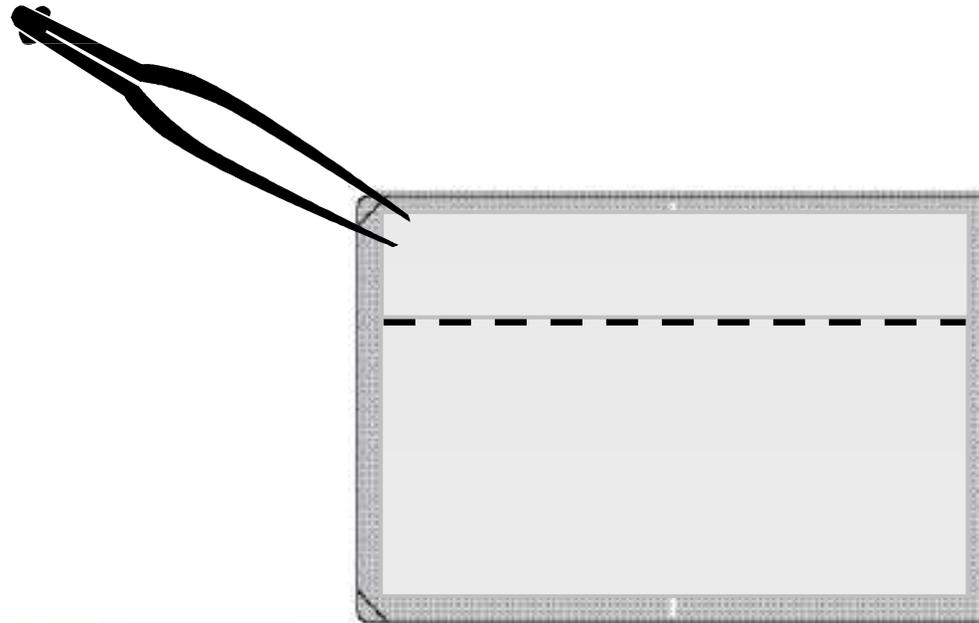
Additional preparation

- Using a razor blade, score the foil covering the 384-well plate around the area to be loaded.



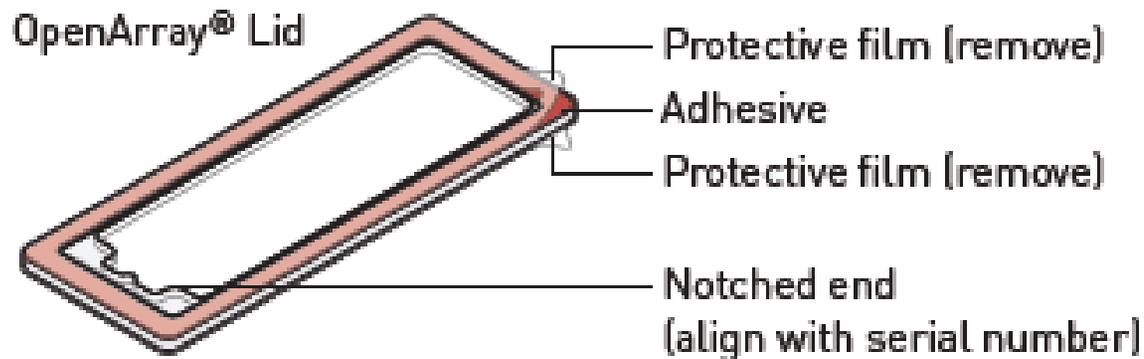
Load plates, remove foil

- Load the 384-Well OpenArray[®] Sample Plate and the OpenArray[®] reaction plate(s) into the AccuFill[™] System.
- Using tweezers or forceps, remove foil from the Sample Plate.



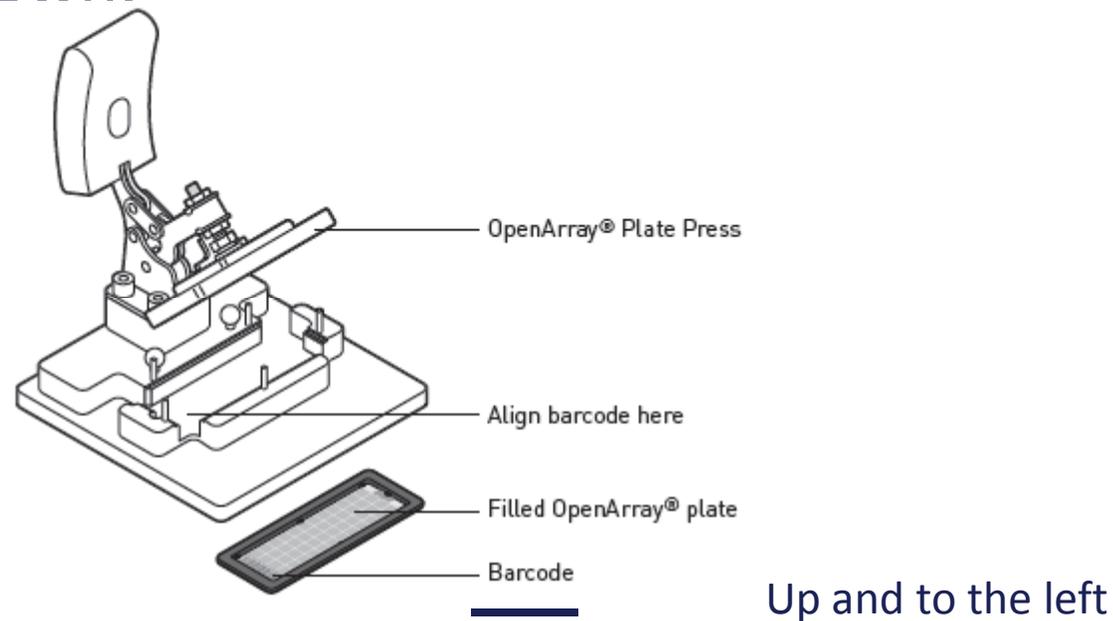
Affix case lids to OpenArray[®] plate

- Take an OpenArray[®] Case Lid.
- Using tweezers or forceps, remove the **protective film** from the adhesive strip (on one side) and the glass cover (other side).



Seal OpenArray® Plate

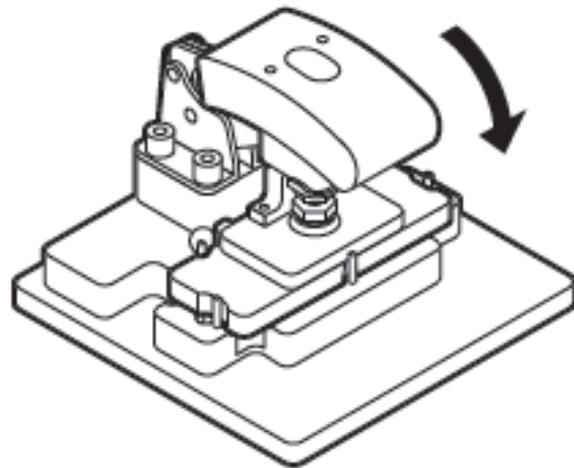
- Set the first OpenArray® reaction plate into the Plate Press, as shown:



- Set the Lid on top

Seal OpenArray® Plate

- Press down firmly for exactly 10 seconds.
- Not too firmly, though.



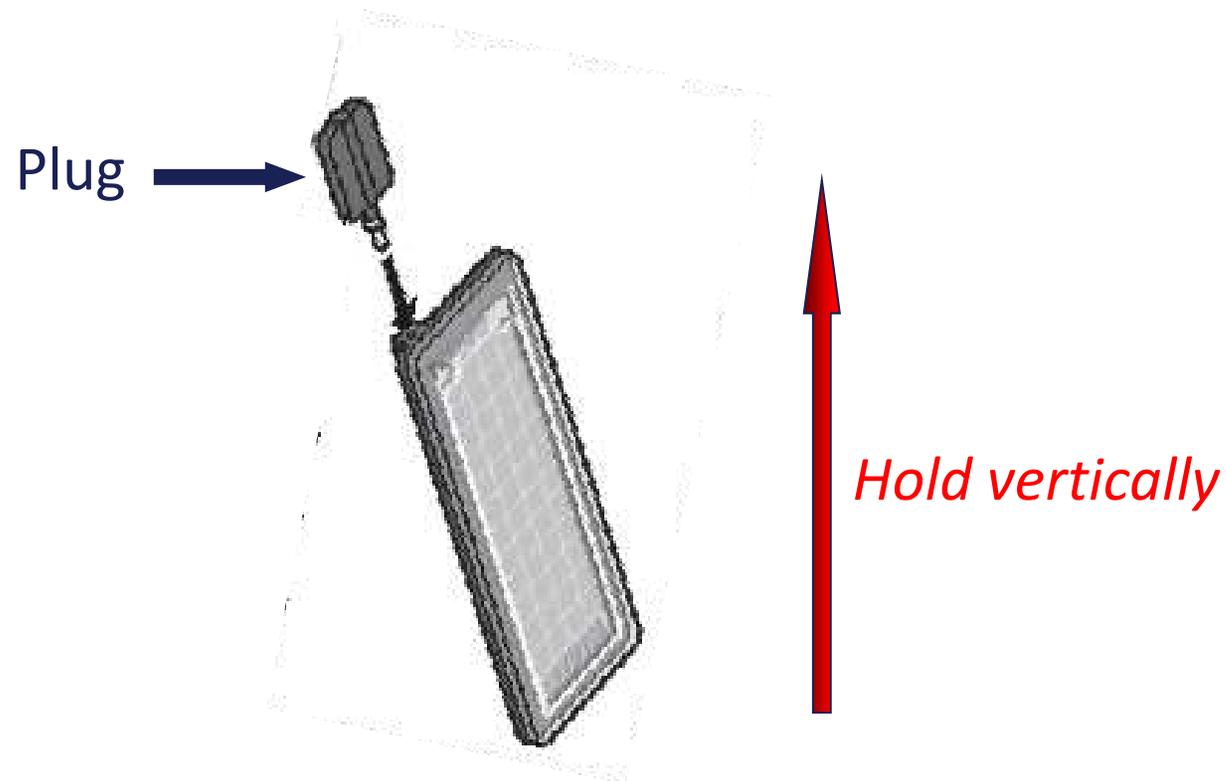
Immersion fluid

- *Slowly* inject fluid from the syringe into the fill port of the sealed case.



Plug the case

- Insert a plug, and twist clockwise until tight.
- Then, remove knob.



Clean Loaded Case

- Wet a lab wipe with EtOH.
- Wipe the case clean, **being careful** not to press too hard on the sides.
- Dry with a clean wipe.
- Change your gloves if immersion fluid on it



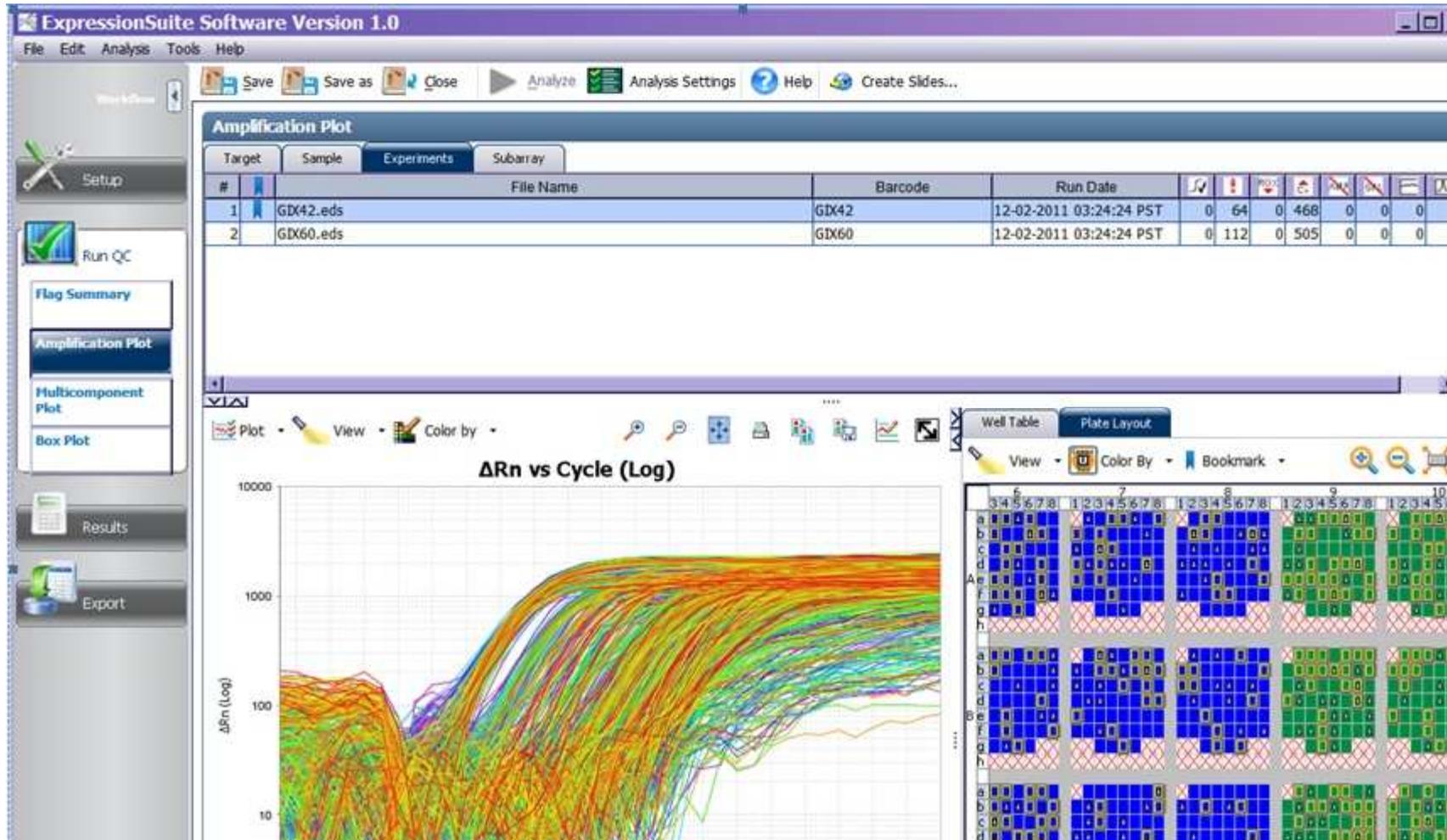
Load into the Instrument

- Load 1-4 OpenArray[®] loaded and sealed plates into the QuantStudio[™] 12K Flex real-time instrument using the dedicated carrier.



Use correct experiment-specific template(s),
and start run
Run plates later after 2 hours
of loading

MicroRNA Data Analyzed using ExpressionSuite™





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