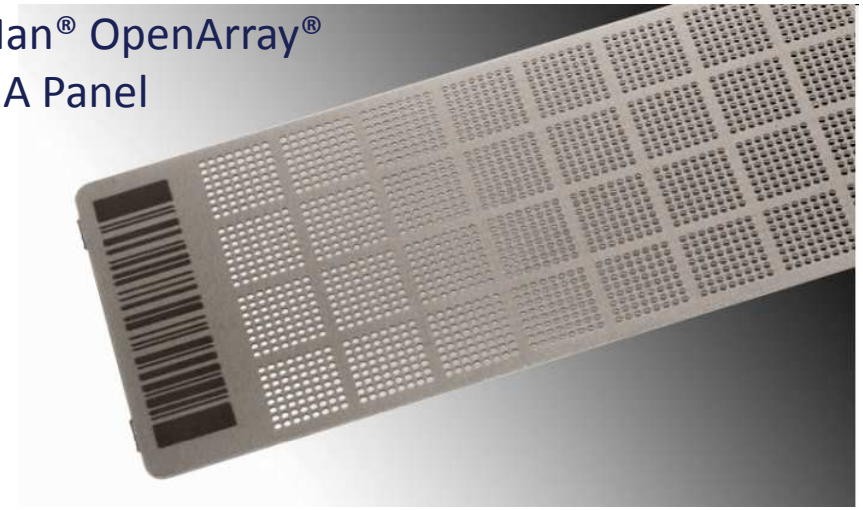
MicroRNA Profiling with TaqMan® OpenArray® Plates

Profiling with TaqMan® MicroRNA Assays

Highly Parallel Assays For miRNA Expression Analysis

Megaplex™
Primer Pools

TaqMan® OpenArray®
miRNA Panel



OpenArray®
Instrumentation

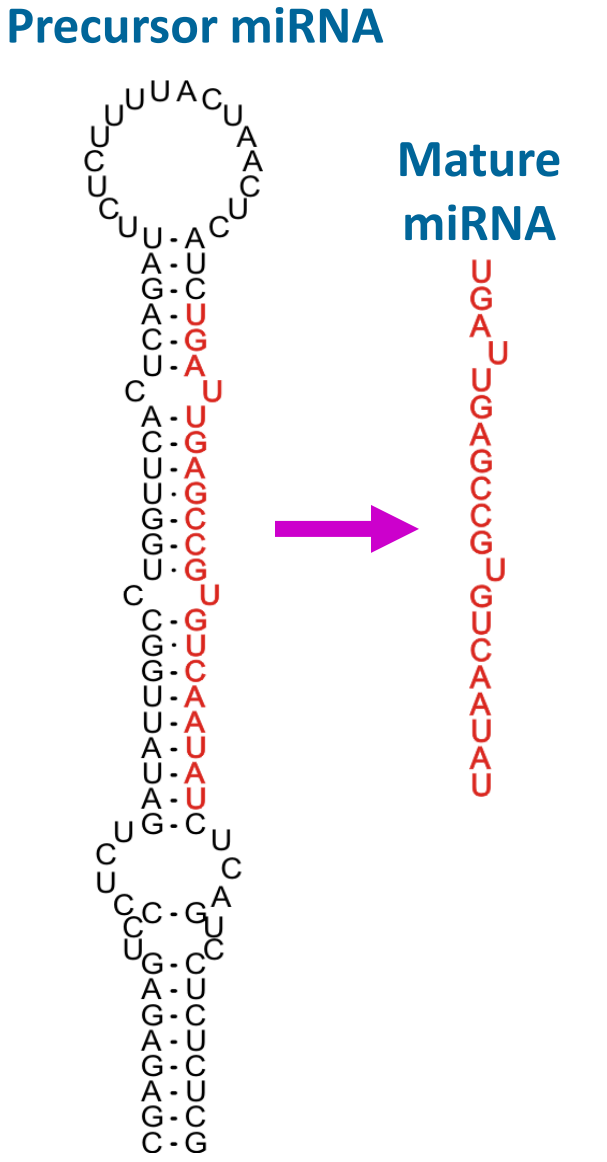
life technologies™

Invitrogen™ | Applied Biosystems® | Gibco® | Molecular Probes® | Novex® | TaqMan® | Ambion® | Ion Torrent™

AB Assay Objective

- Develop a Real-Time PCR TaqMan® Assay to quantify mature* microRNAs

**Mature miRNAs are the biologically active form*



TaqMan® MicroRNA Assays Design

Convert to cDNA

Quantitate microRNA Expression

life technologies™

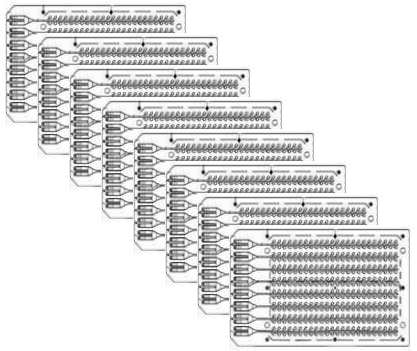
Invitrogen™ Applied Biosystems® Gibco® Molecular Probes® Novex® TaqMan® Ambion® Ion Torrent™

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?

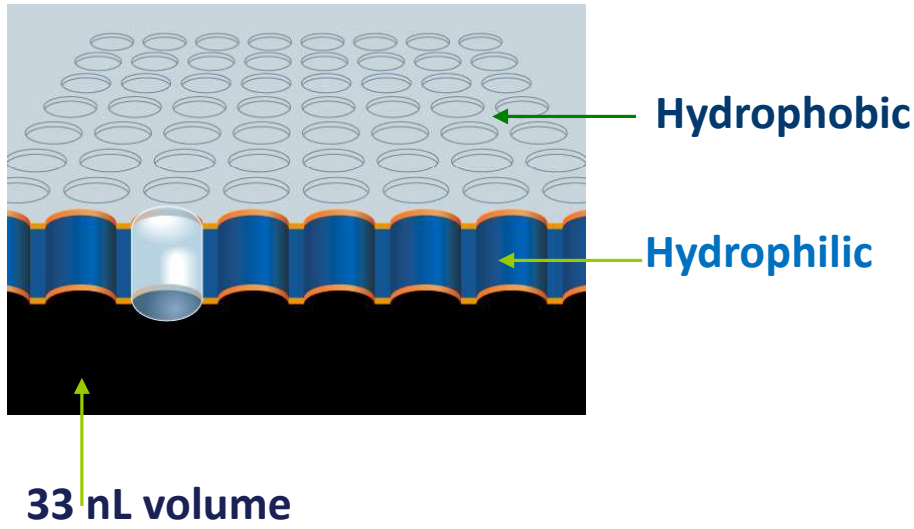
- Profile well-characterized and well-studied miRNAs for Cancer biomarker discovery research, MicroRNA regulation of mRNA gene expression, and MicroRNA functional analysis

Performance

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

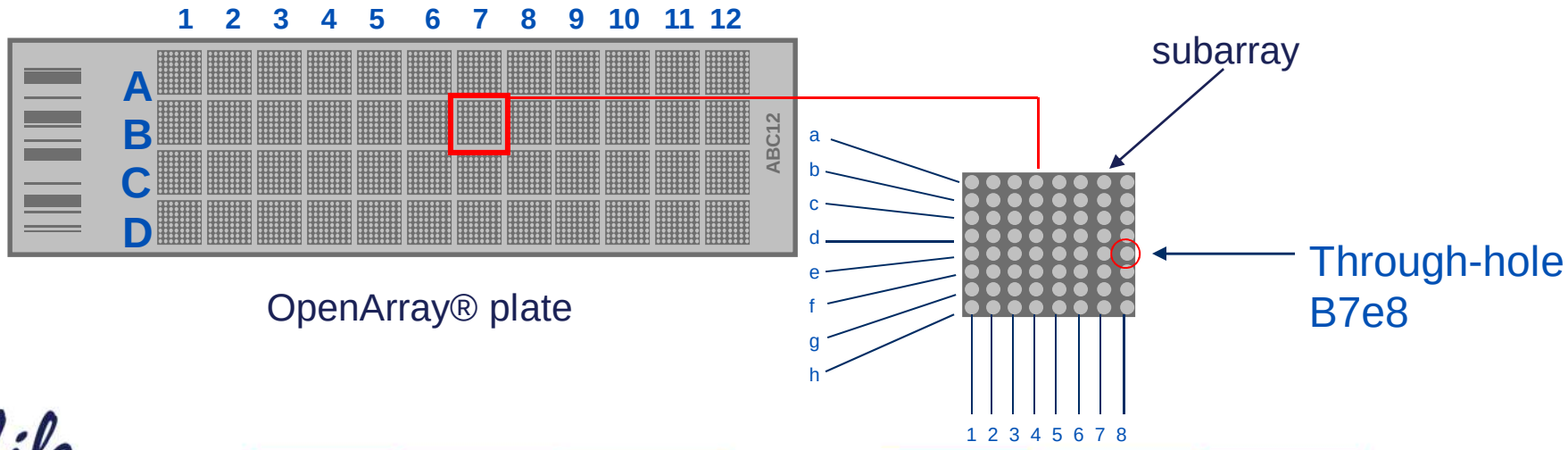
OpenArray® plate Anatomy

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



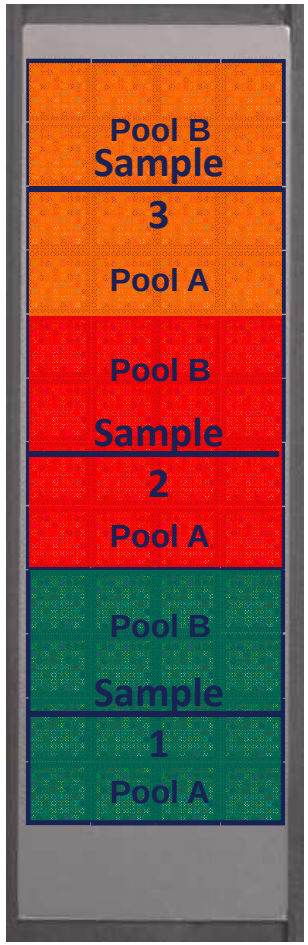
What is an OpenArray® plate?

- 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

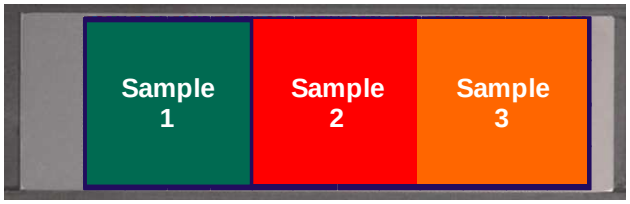
- Broad coverage
  - Run three samples per OpenArray® plate
  - 818 assays per sample (758 unique assays)
    - > 3 positive and 1 negative/process control per subarray
- High-throughput
  - 12 samples per ~2.5 hr instrument run
  - 48 samples per 8hr working day
- Cost effective
  - Rapid sample screening 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

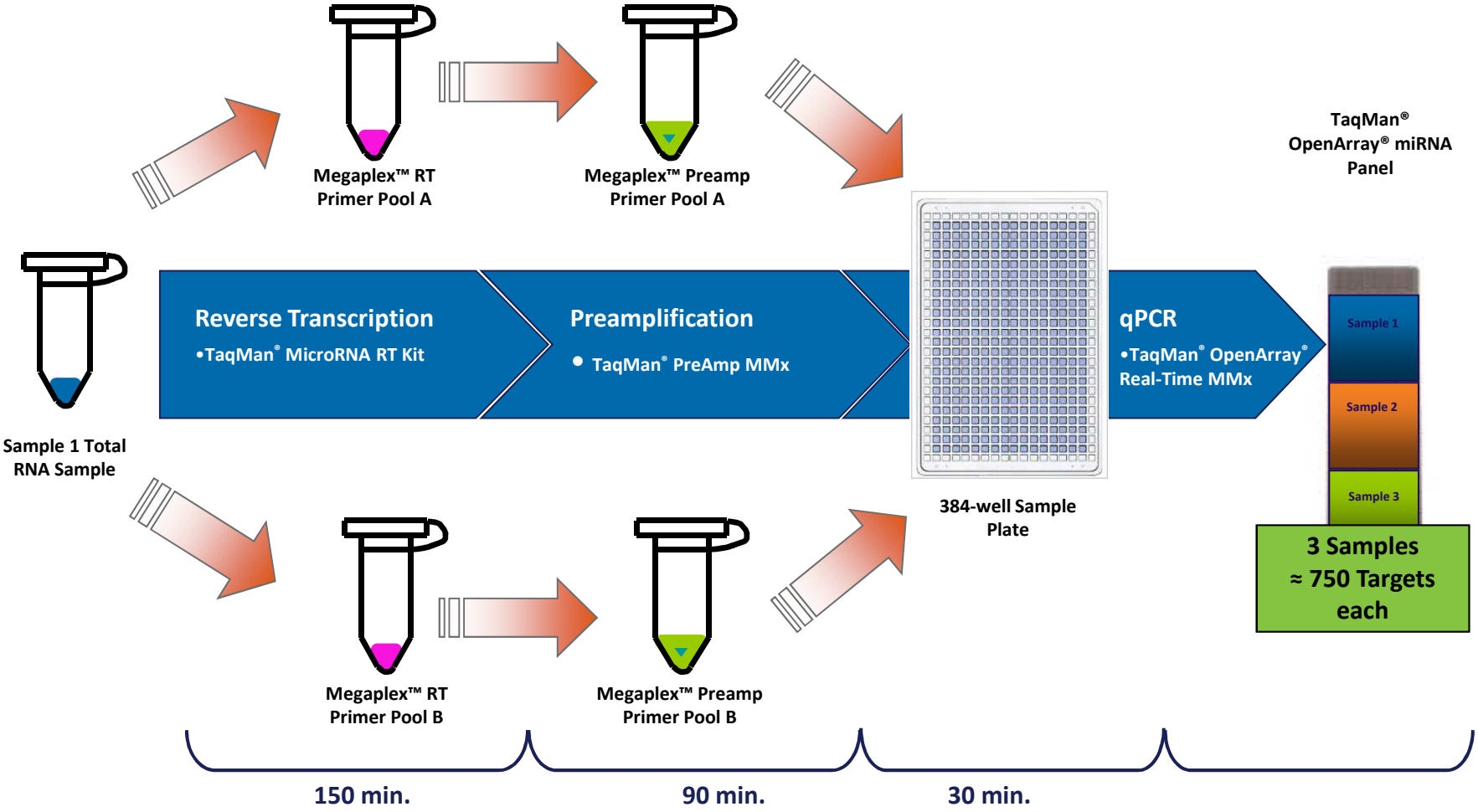
- Minimum order size 1 panel
- Inventoried
- 2-3 days shipping
- P/N 4470187



OpenArray® Rodent MicroRNA Panel QuantStudio™ 12K Flex

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

OpenArray® miRNA Profiling Workflow



Total time < 8 hours

150 min.*
(*up to 4 arrays/12 samples run simultaneously)

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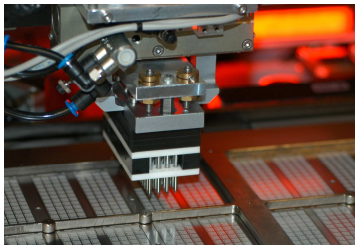
OpenArray® Block User Workflow Overview

At Life Technologies

TaqMan® assays ordered on-line



The assays are spotted on the OpenArray® plate

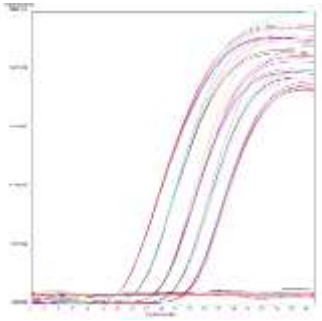
OpenArray® plates staked in case

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!

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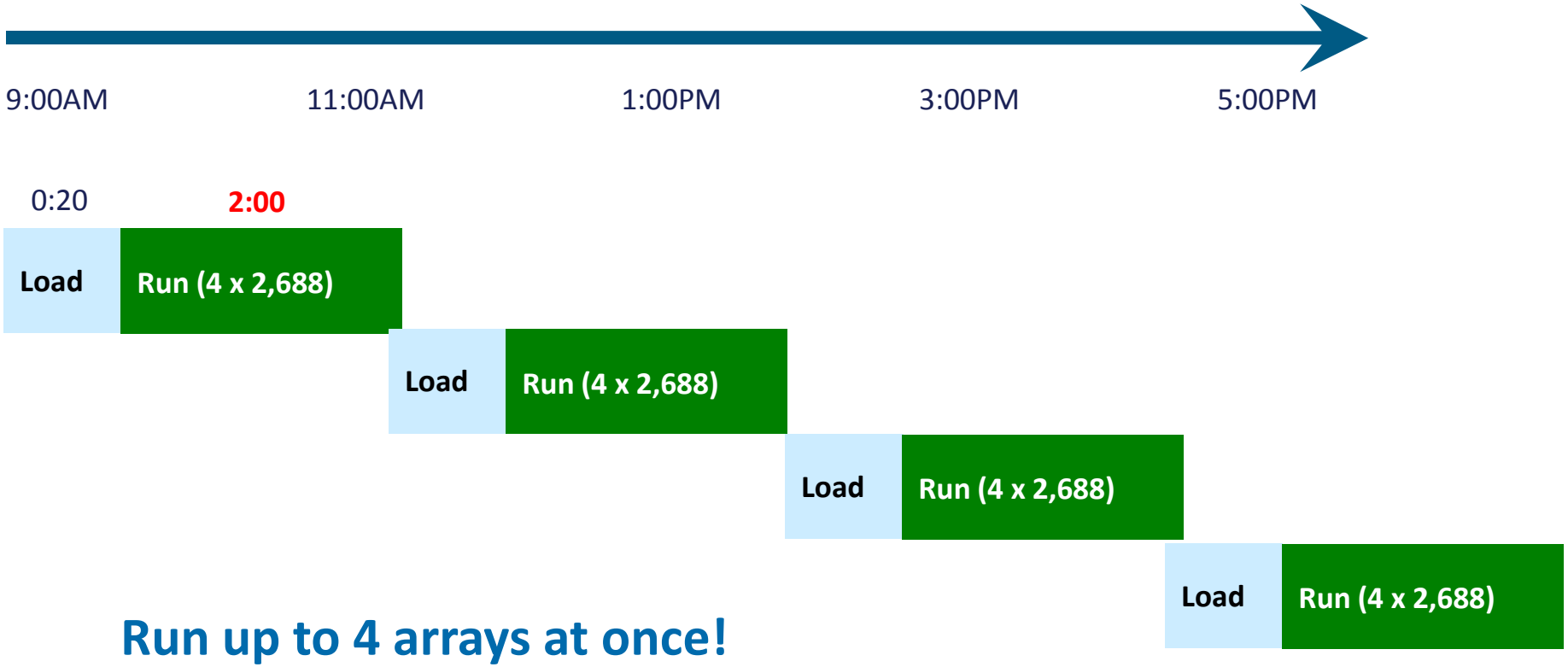
Invitrogen™ | Applied Biosystems® | Gibco® | Molecular Probes® | Novex® | TaqMan® | Ambion® | Ion Torrent™

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



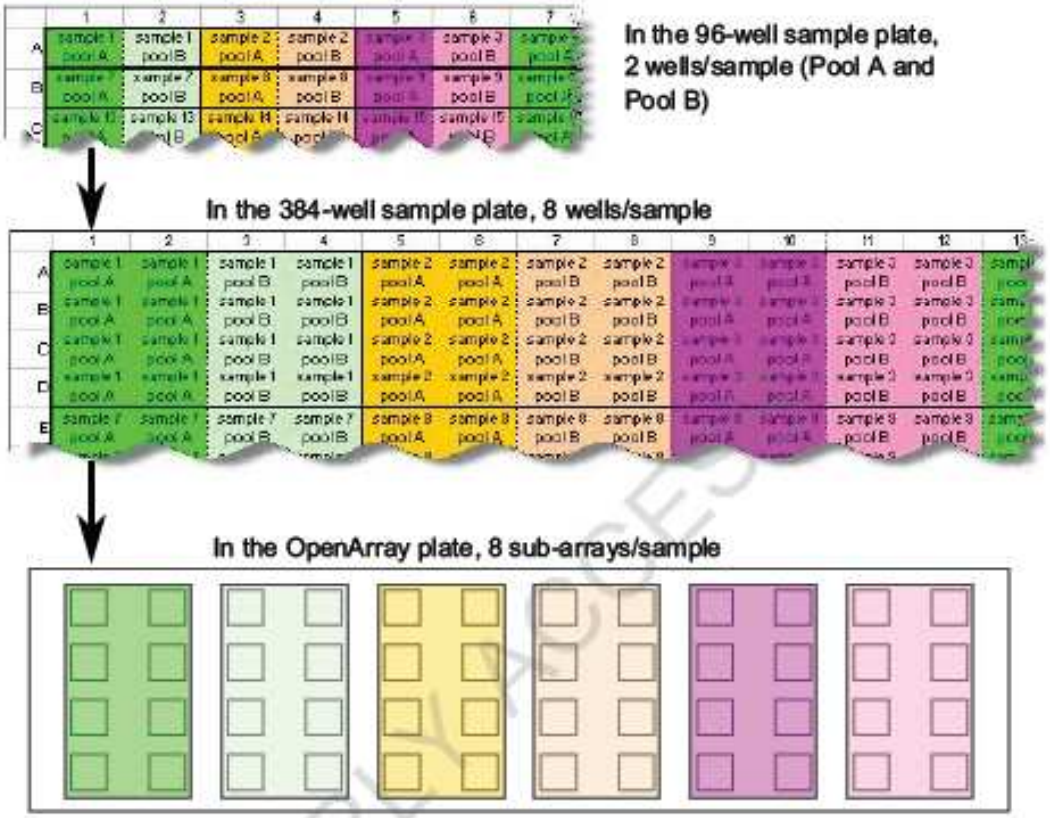
Run up to 4 arrays at once!
16 arrays/ day
>43,000 reactions, 1 operator

life technologies™

Invitrogen™ Applied Biosystems® Gibco® Molecular Probes® Novex® TaqMan® Ambion® Ion Torrent™

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.

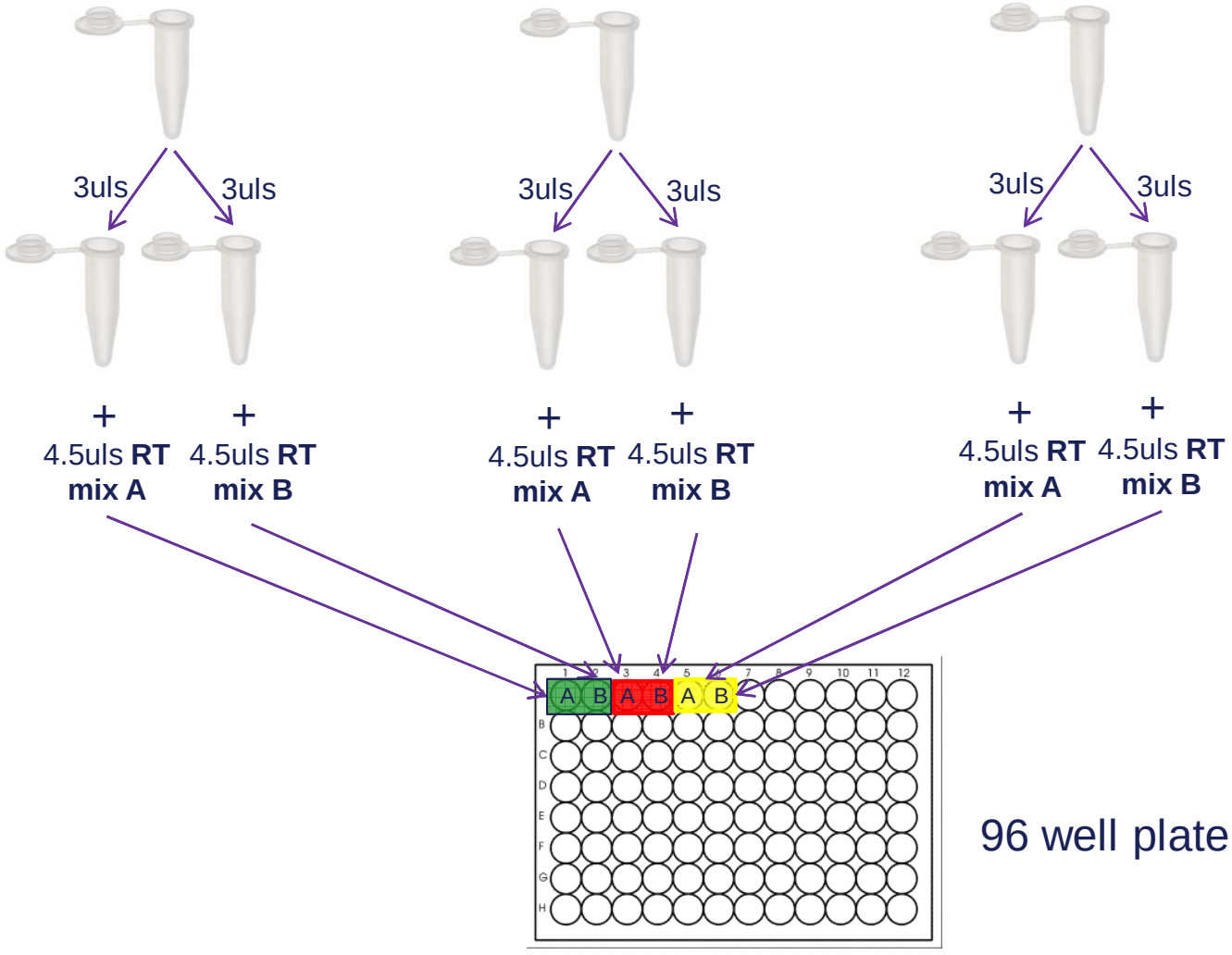
STOPPING POINT If needed, you can store the RT product (cDNA) at -15 to -25°C for up to 1 month.

Step 1 : Megaplex™ RT reaction: 96 well plate

Sample 1
33ngs/ul and ≥6uls

Sample 2
33ngs/ul and ≥6uls

Sample 3
33ngs/ul and ≥6uls



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

add

2.5 uls **RT reaction A from Step 1**

2.5 uls **RT reaction B from Step 1**

For sample 2 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**

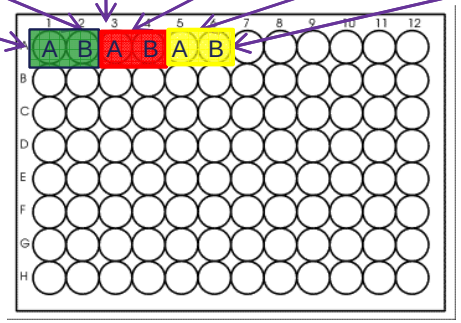
For sample 3 prepare pre-amp mmix A and B

22.5 uls 22.5 uls

add

2.5 uls **RT reaction A from Step 1**

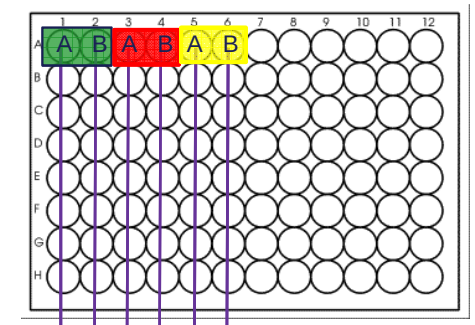
2.5 uls **RT reaction B from Step 1**



96 well plate (2nd)

Pre-amp reaction ~1.5h

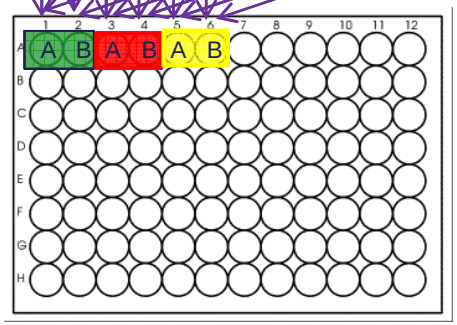
Step 3 :Dilute the Pre-amplification Product



96 well plate from previous step

4µL + 156µL

Preamplified cDNA 0.1x TE pH 8.0

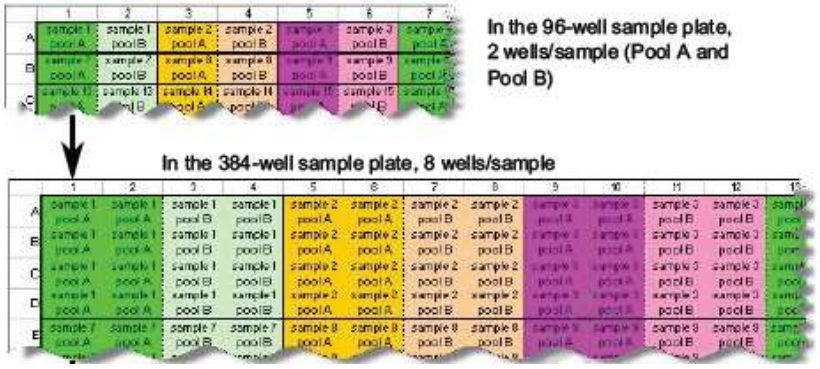


96 well plate (3nd)

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 µL 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 µL of diluted Pool A PreAmp and 22.5 µL of diluted Pool B PreAmp into the other well.
3. Transfer 5 µl of PCR mix in sample plate.

Step 4: prepare PCR reaction

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

add

22.5 uls of diluted pre-amplified sample from step 3

22.5 uls of diluted pre-amplified sample from step 3

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

add

22.5 uls of diluted pre-amplified sample from step 3

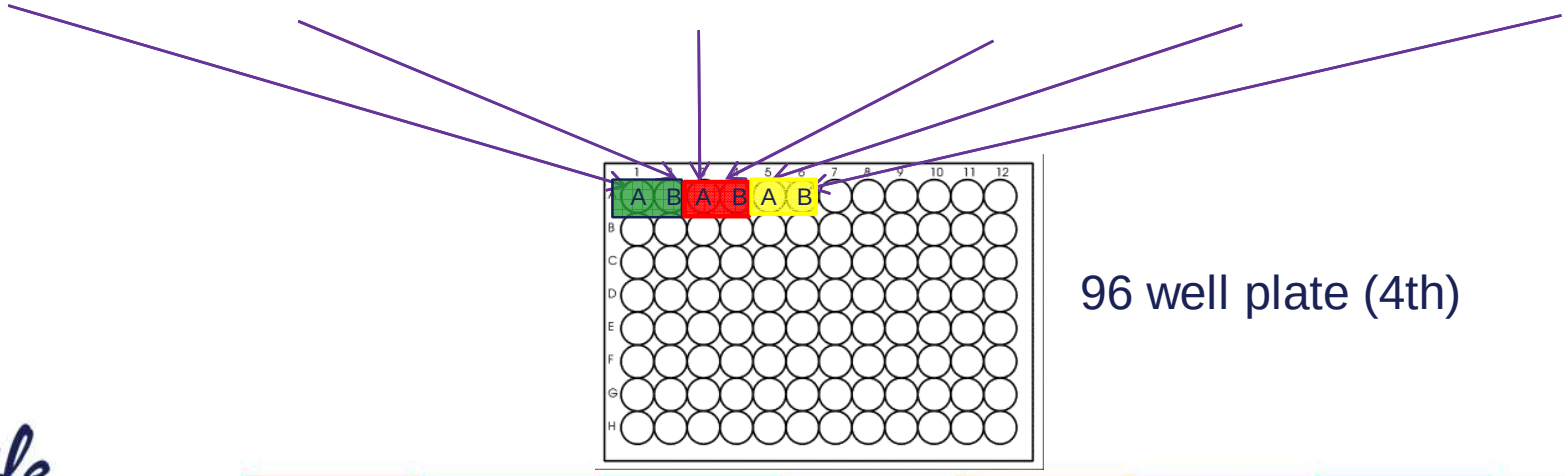
22.5 uls of diluted pre-amplified sample from step 3

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

add

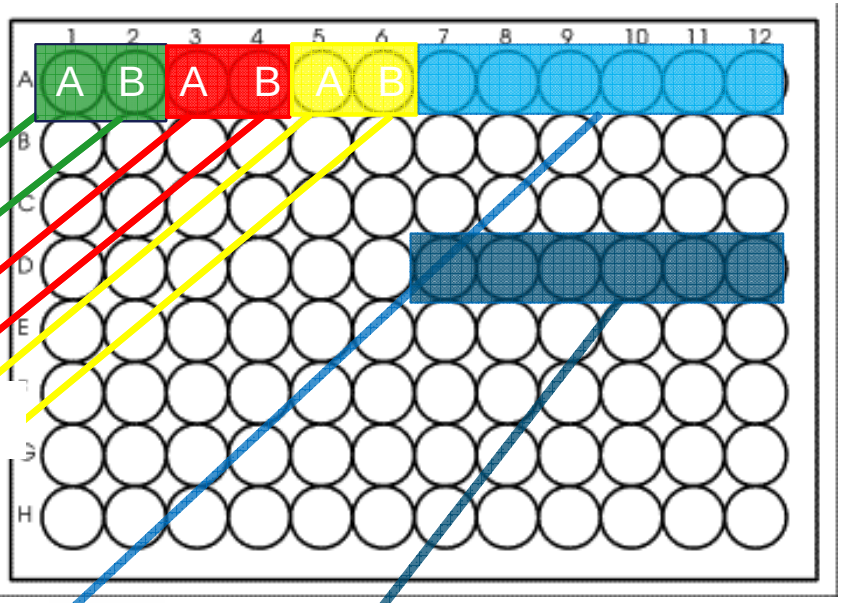
22.5 uls of diluted pre-amplified sample from step 3

22.5 uls of diluted pre-amplified sample from step 3



96 well plate (4th)

96 well plate from previous step



5uls in each well

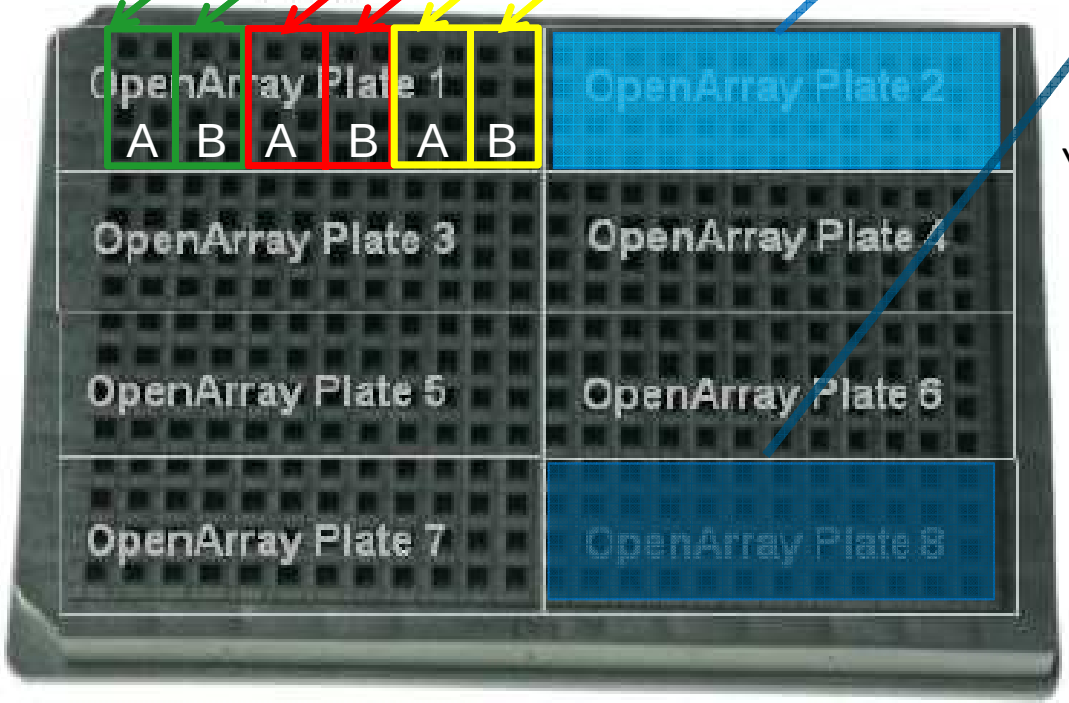
1-12

A-D

E-H

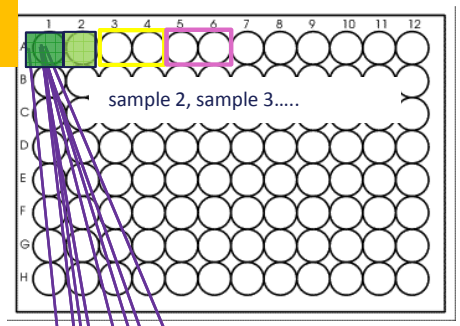
I-L

M-P



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

45uls in each well

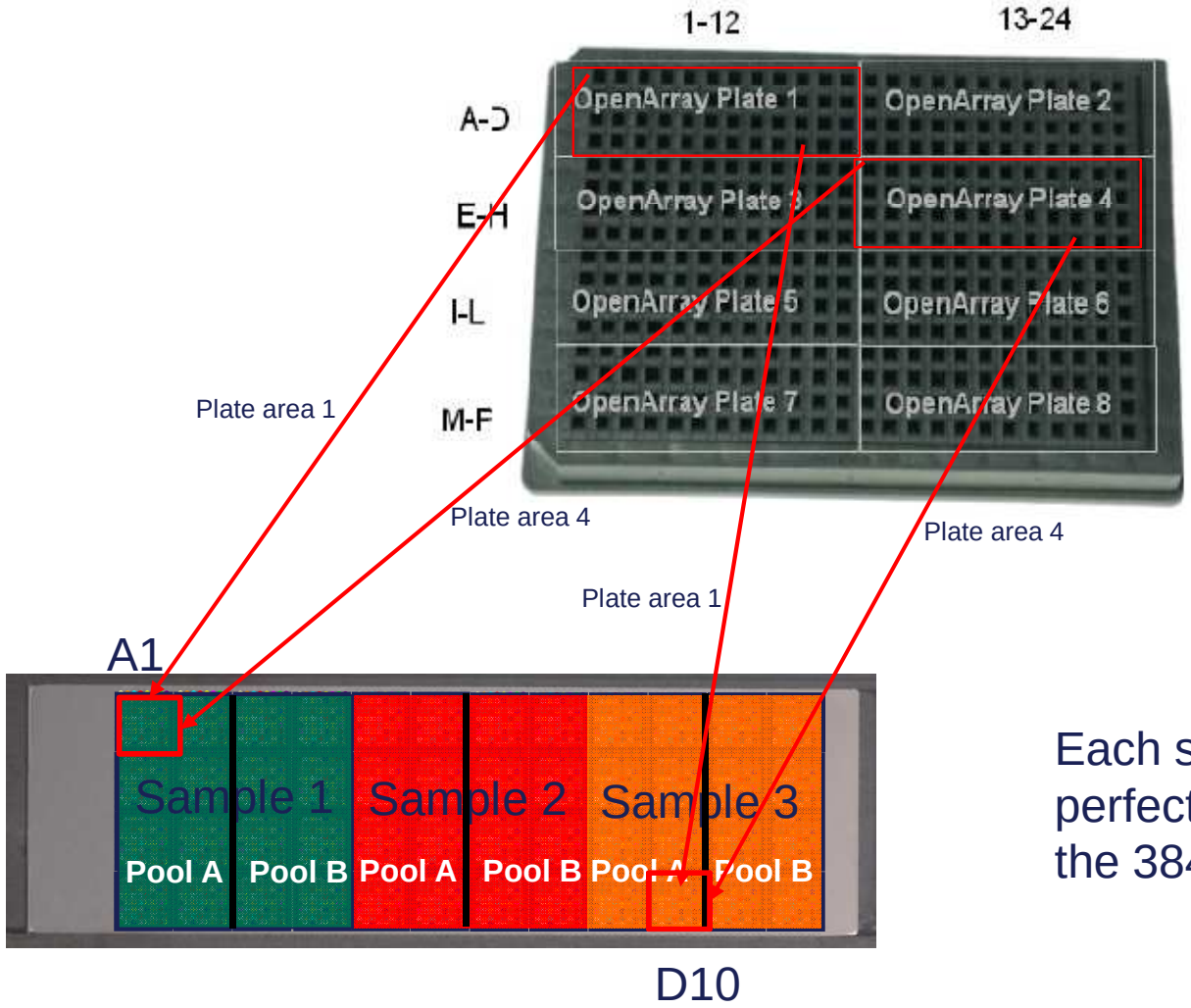


TaqMan® OpenArray® 384-Well Sample plate

5µls 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	sampl pool
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	samp pool
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	samp pool
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	samp pool
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	samp pool

In the OpenArray plate, 8 sub-arrays/sample

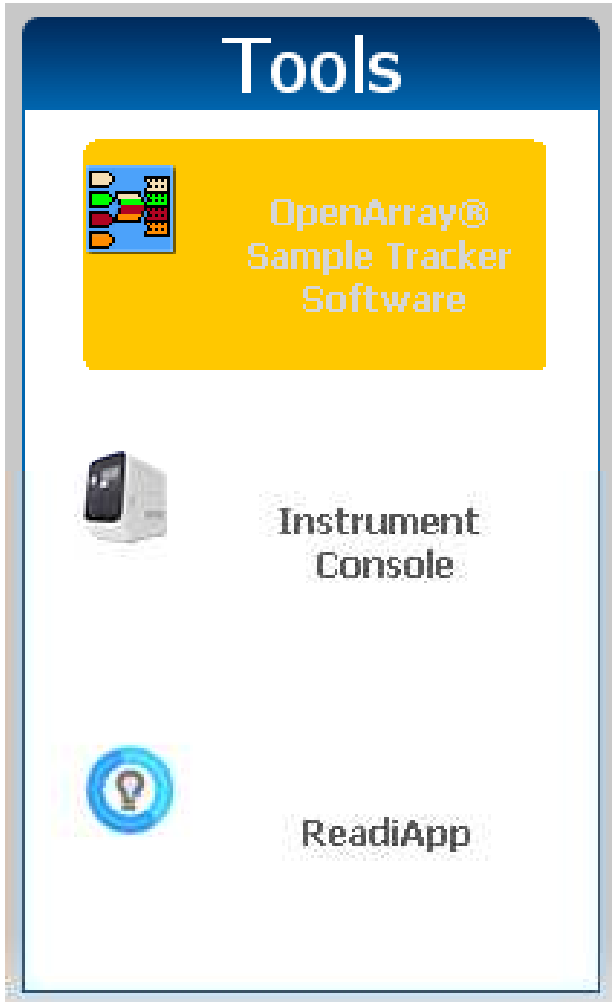


Each subarray overlaps perfectly with a single well in the 384 well sample plate

SampleTracker

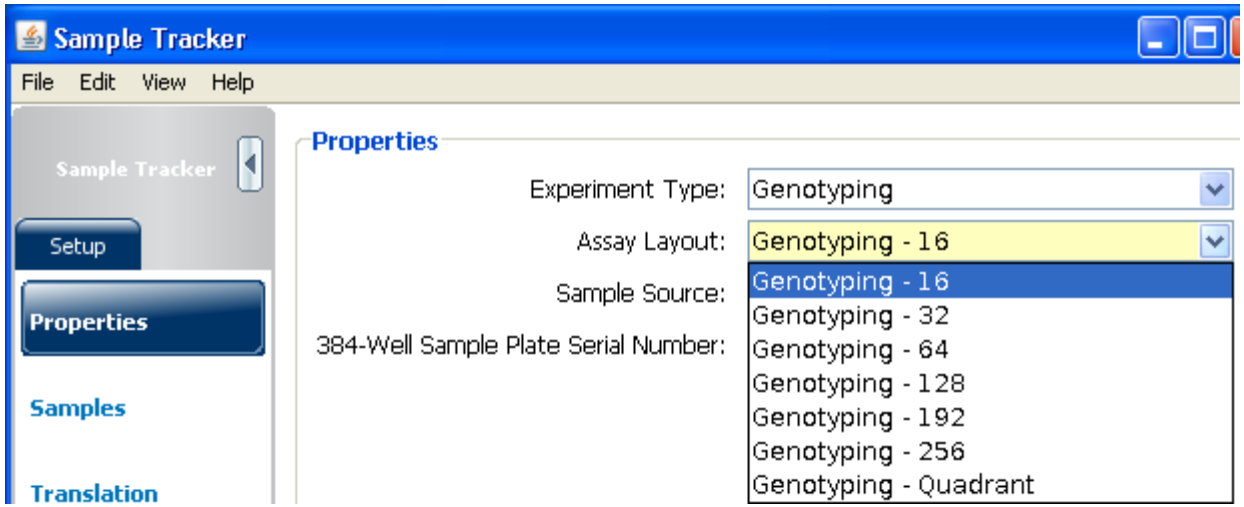
Sample Tracker

- 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.

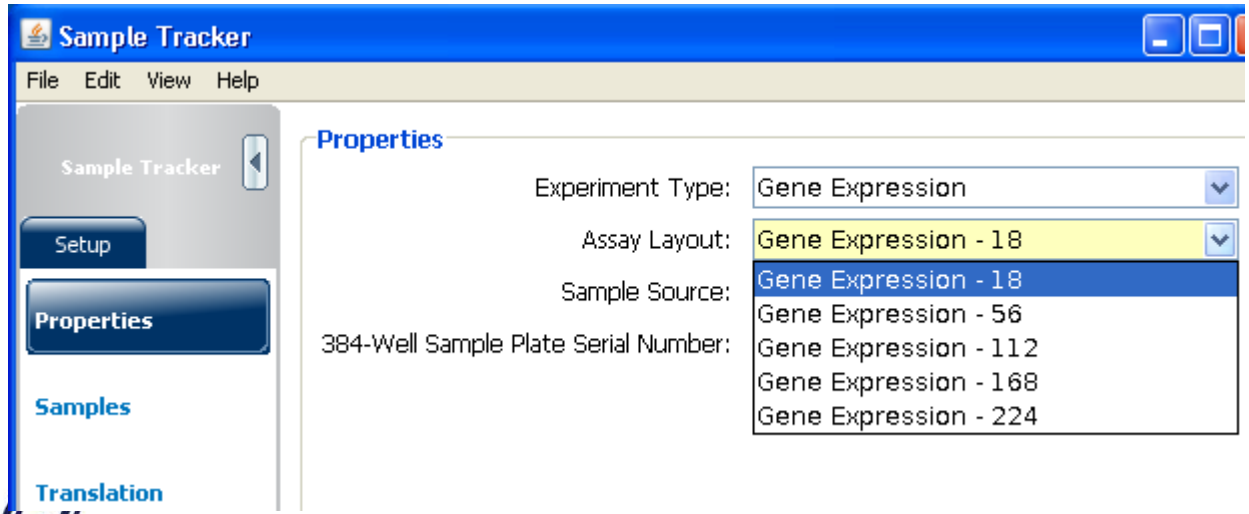


Sample Tracker – Properties

- Under Properties menu - select experiment type and assay layout



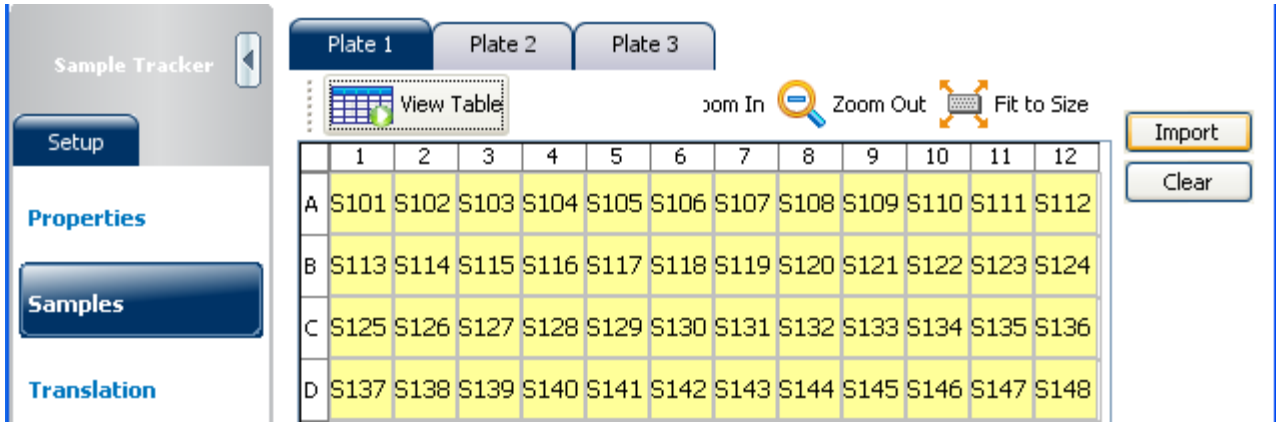
Genotyping



Gene Expression

Sample Tracker - Samples

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

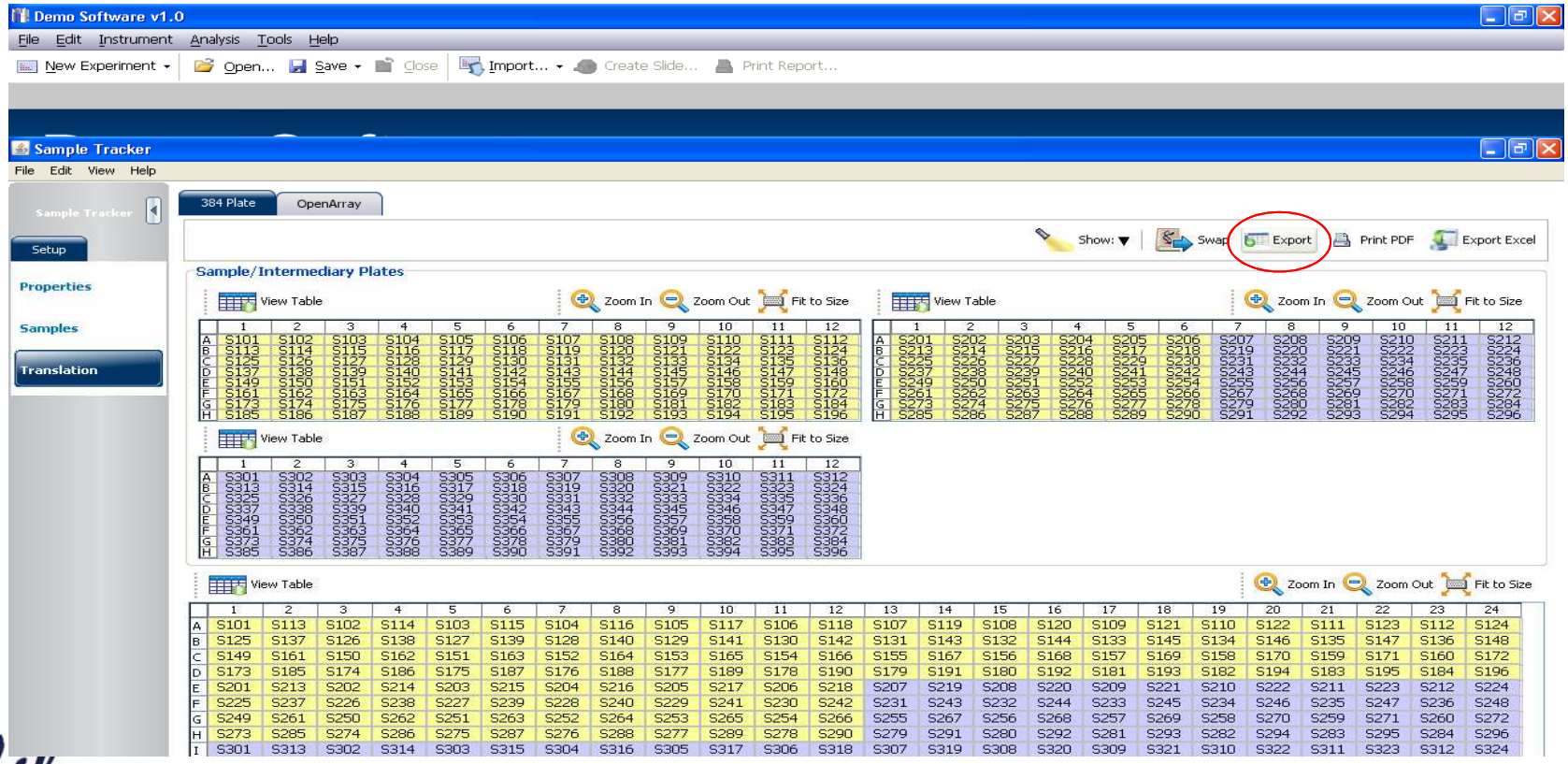


- Input .csv file format for 96-well plates:
  - Files are created by user

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

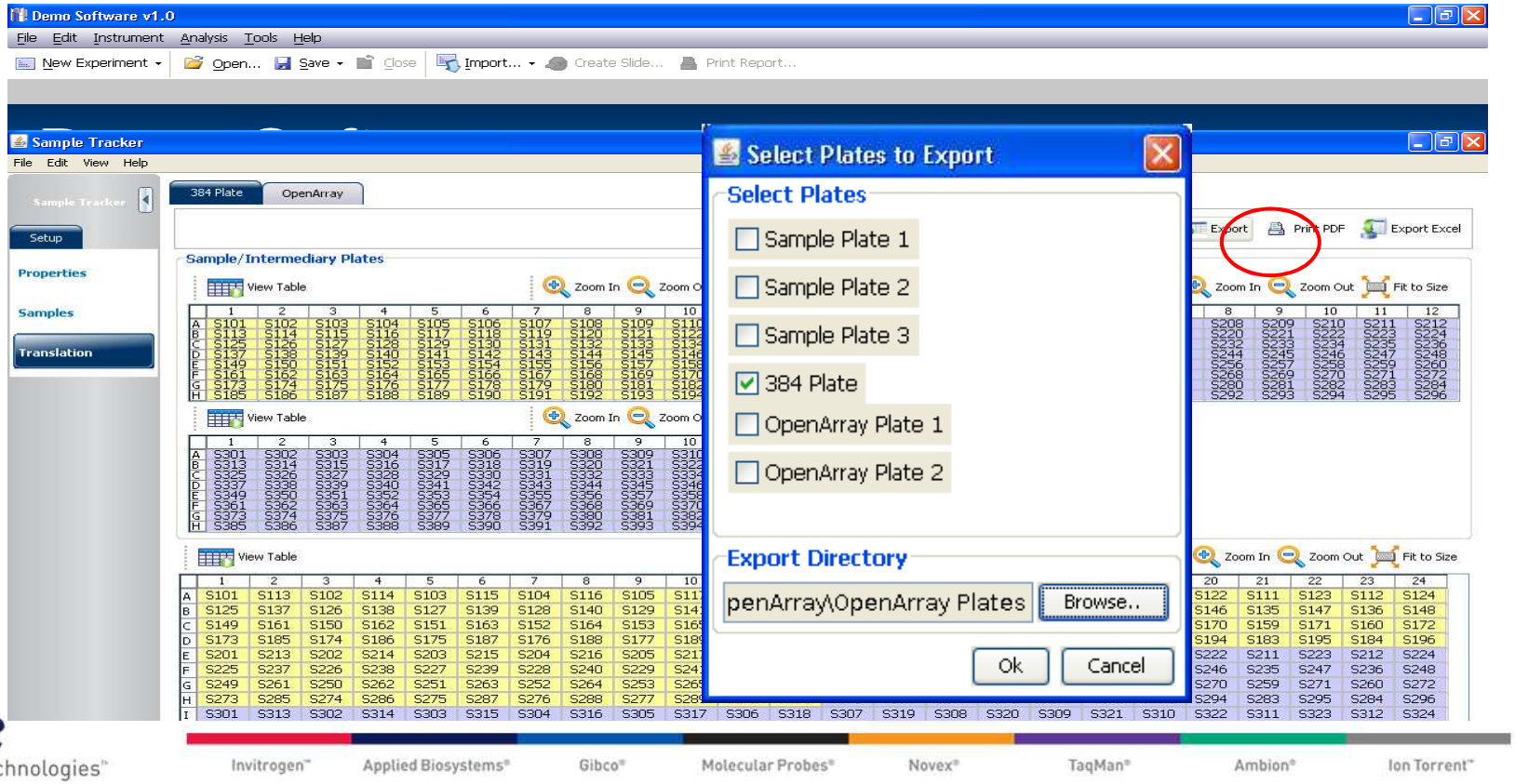
Sample Tracker - Translation

- 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)



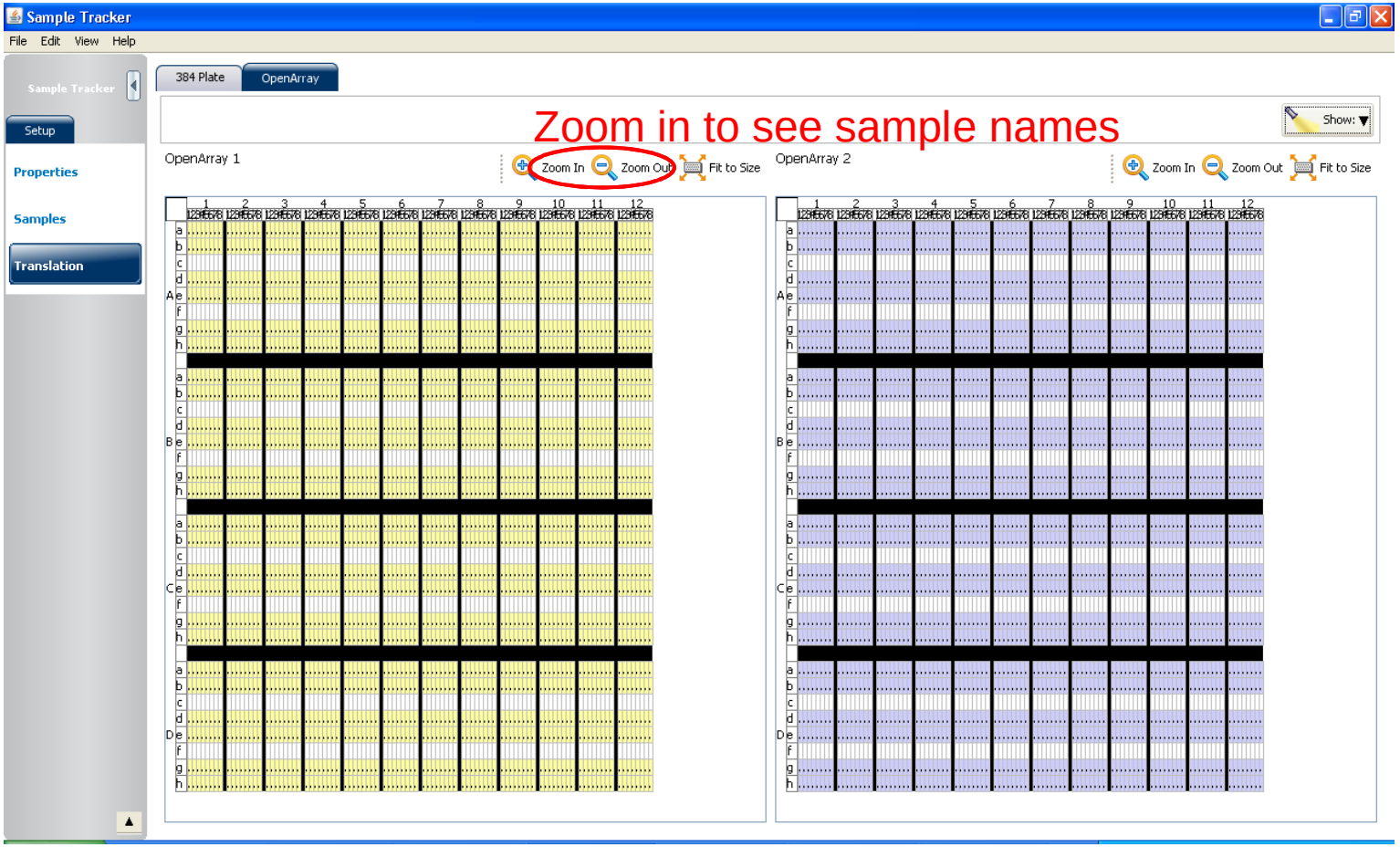
Sample Tracker - Translation

- 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.



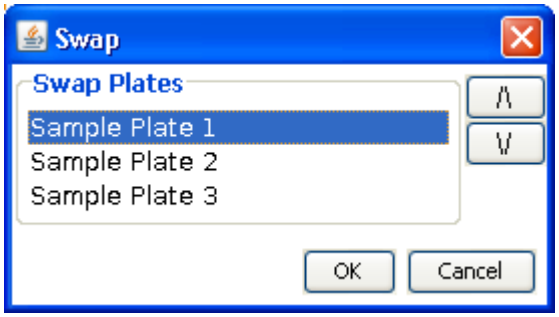
Sample Tracker - Translation

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

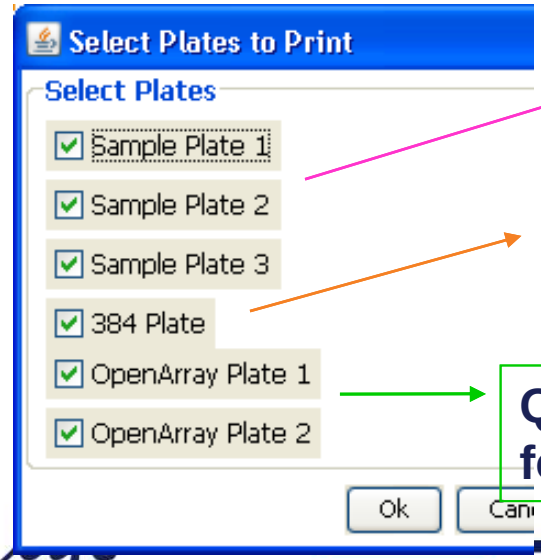


Sample Tracker – Translation Features

- 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

.edt

.las

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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

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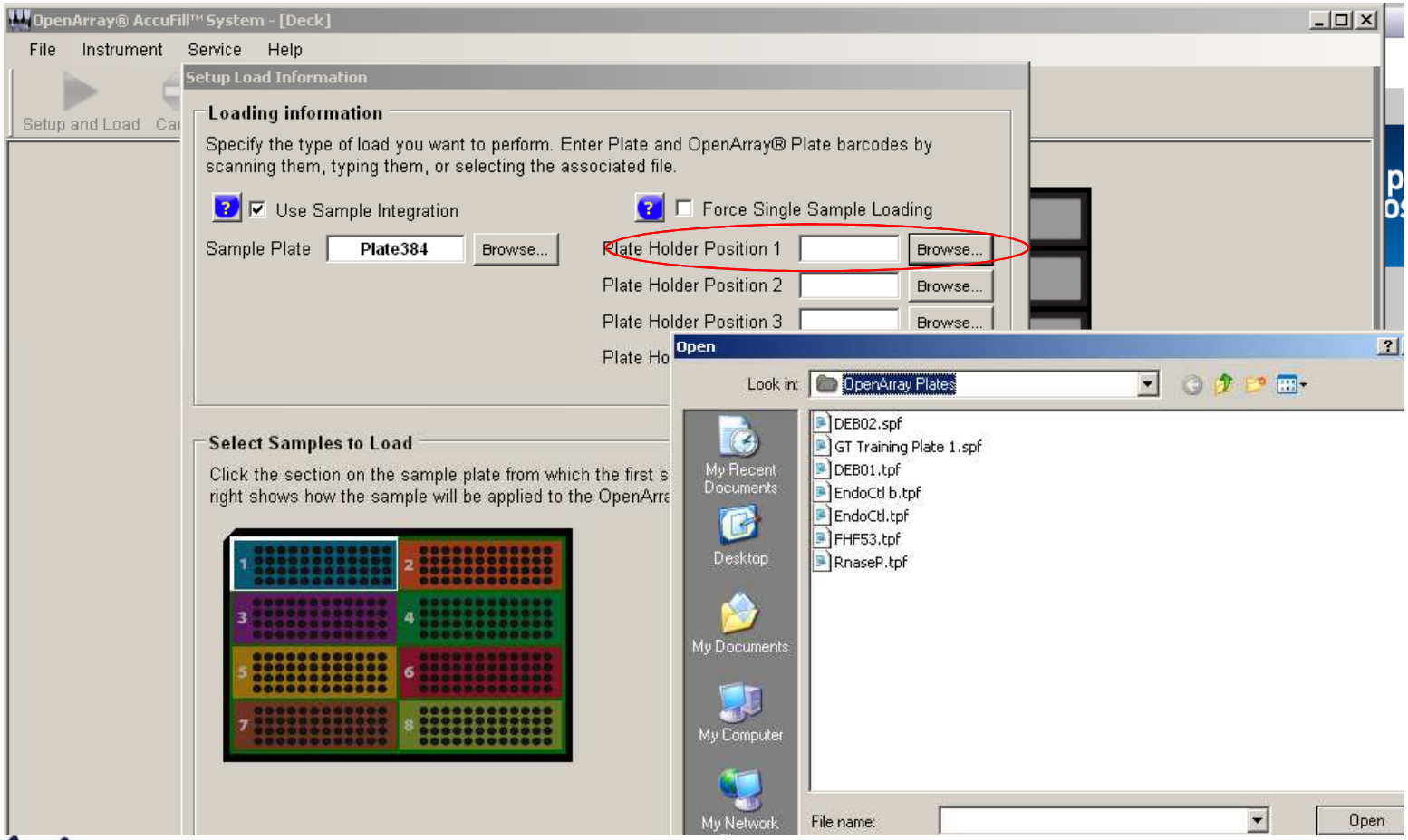
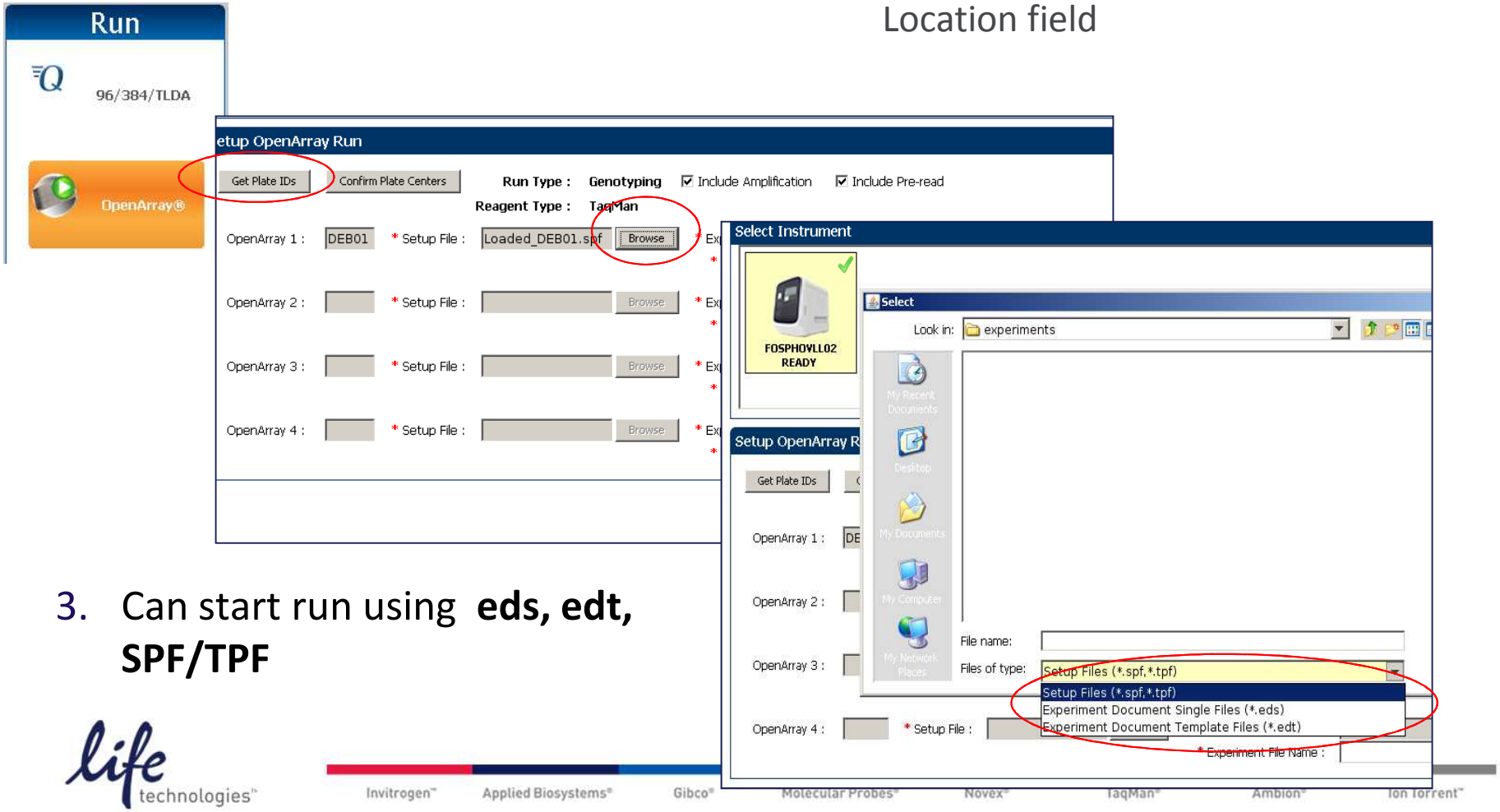
In AccuFill™ System software, v1.x

- Integrate 384W sample plate + tpf.spf



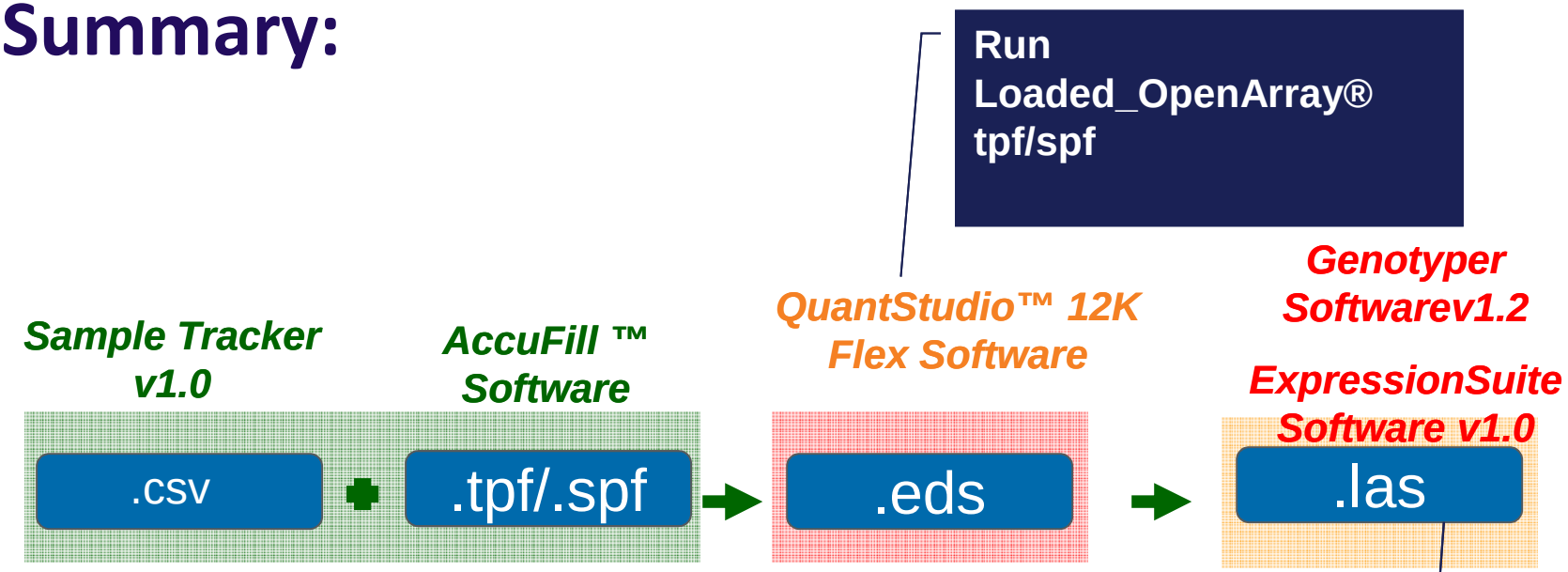
Starting OpenArray® Run in QuantStudio™ 12K Flex Software

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



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

In Summary:



life technologies™

Invitrogen™ Applied Biosystems® Gibco® Molecular Probes® Novex® TaqMan® Ambion® Ion Torrent™

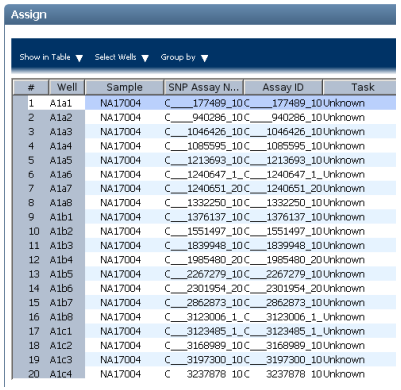
Why are we doing this?

- 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

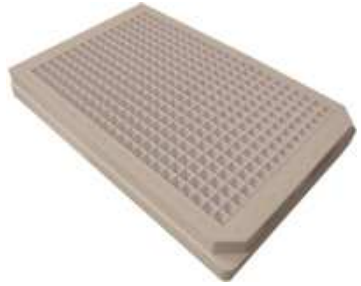
3072-well OpenArray® plate: for cycling and end-point read



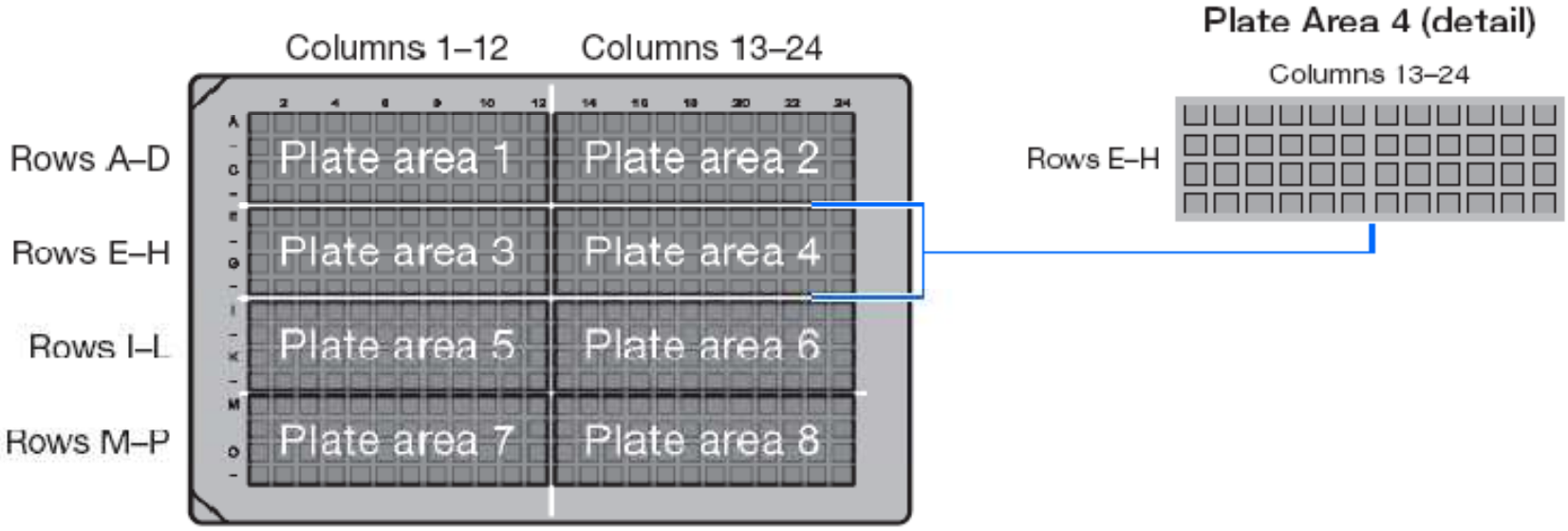
QuantStudio™ Software: data analysis

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



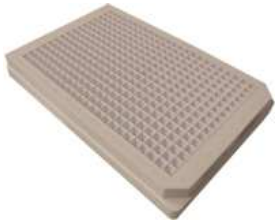
Sample Loading using the OpenArray® AccuFill™ System

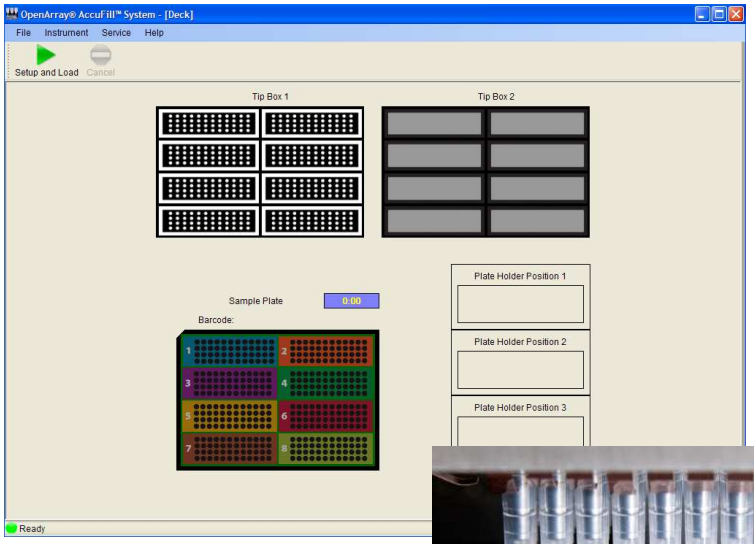
Prepare 384-well sample plate

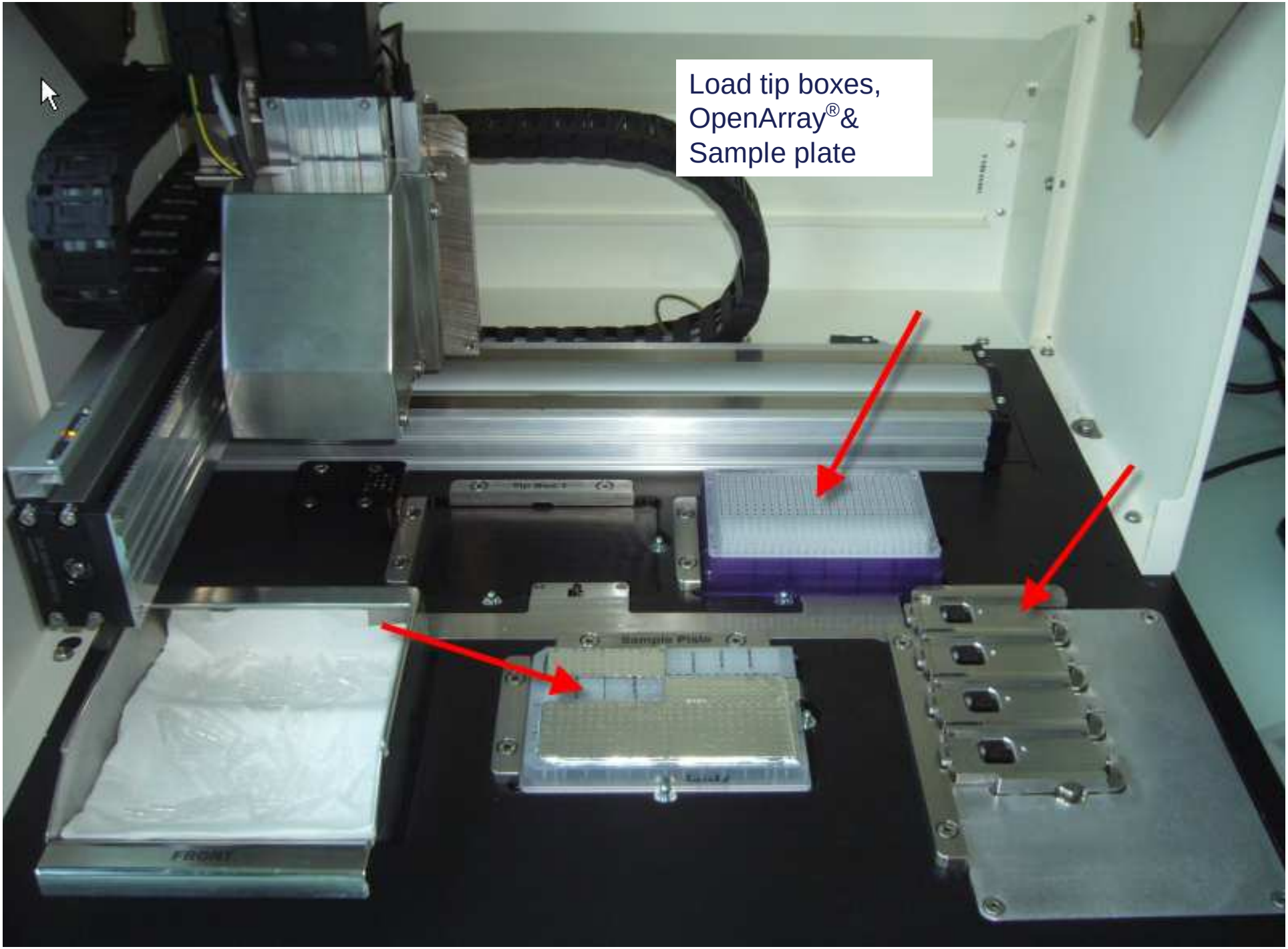
Load tip boxes, OpenArray® & Sample plate

Choose program and press "start"

Remove loaded OpenArray® Plate





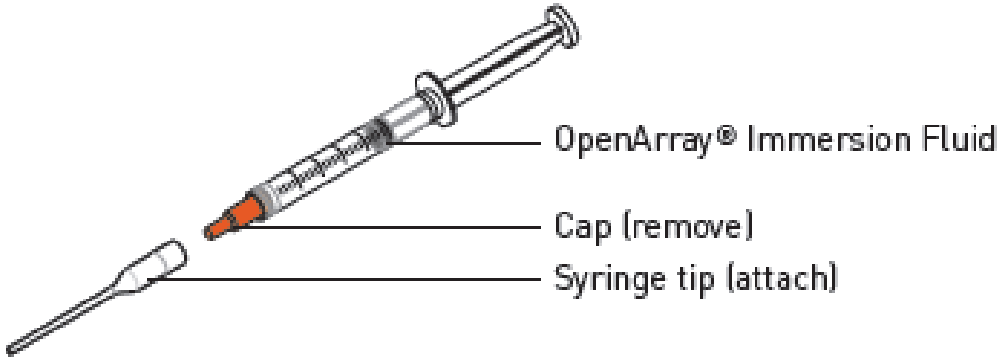

Load tip boxes,
OpenArray®&
Sample plate

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.

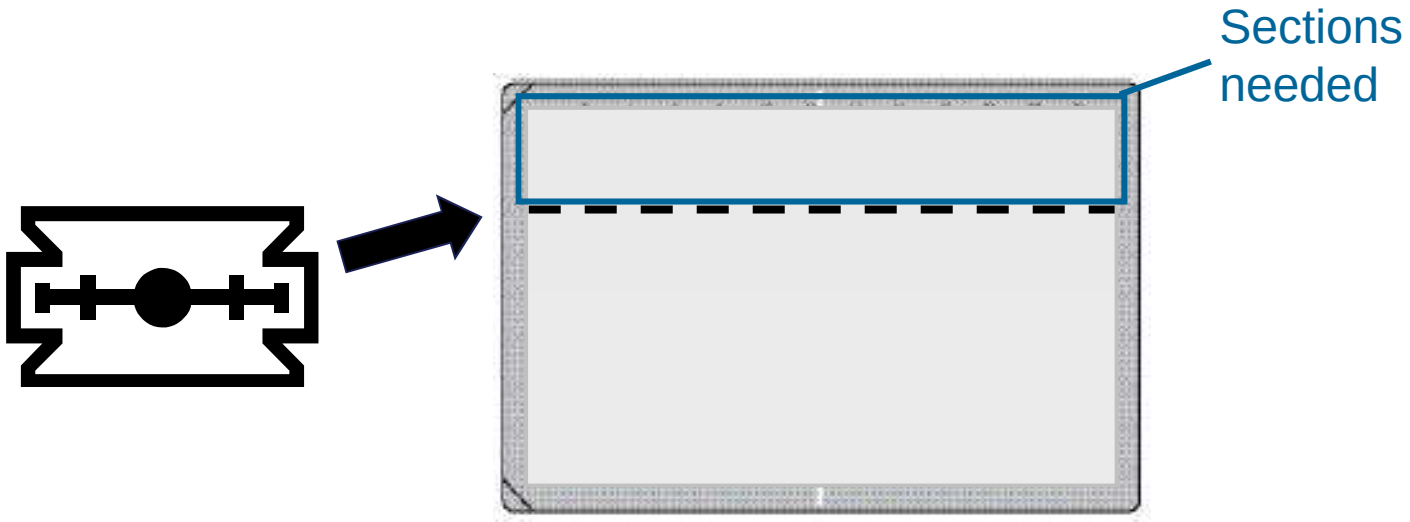
Additional preparations

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



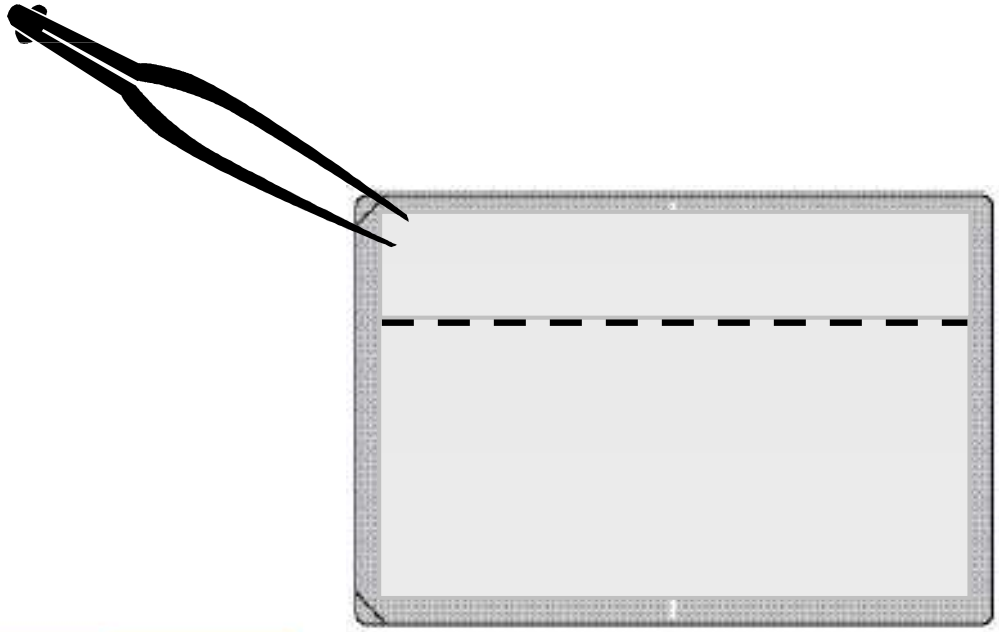
Additional preparation

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



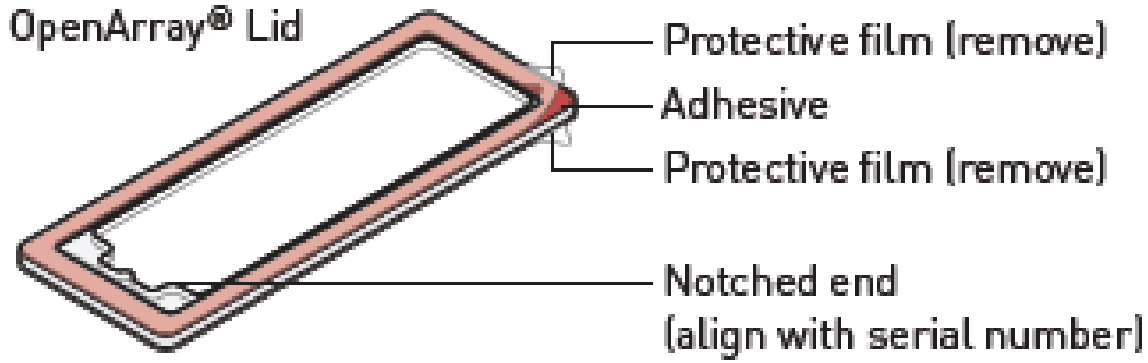
Load plates, remove foil

- 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.



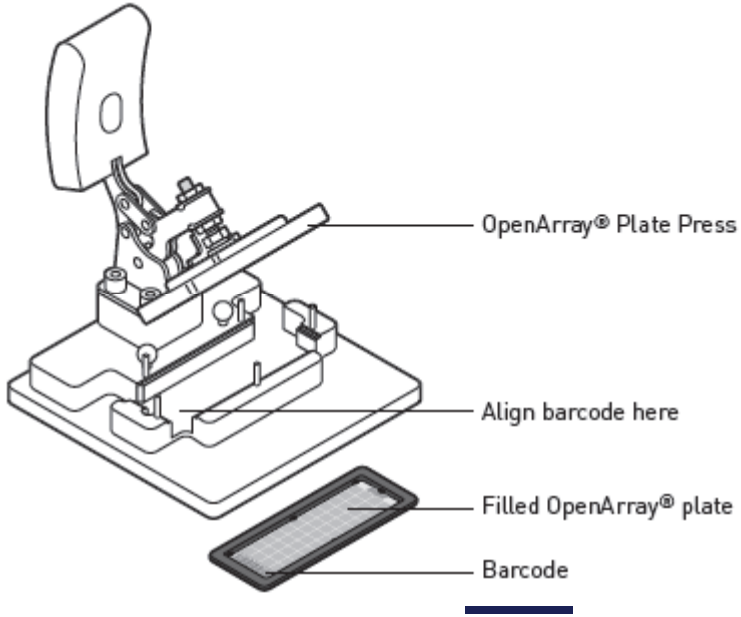
Affix case lids to OpenArray® plate

- 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:

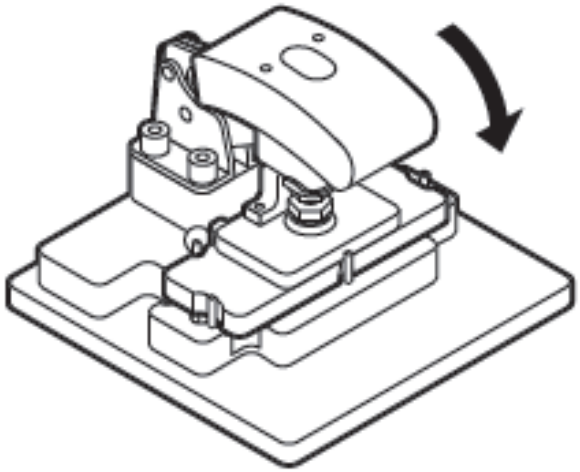


Up and to the left

- Set the Lid on top

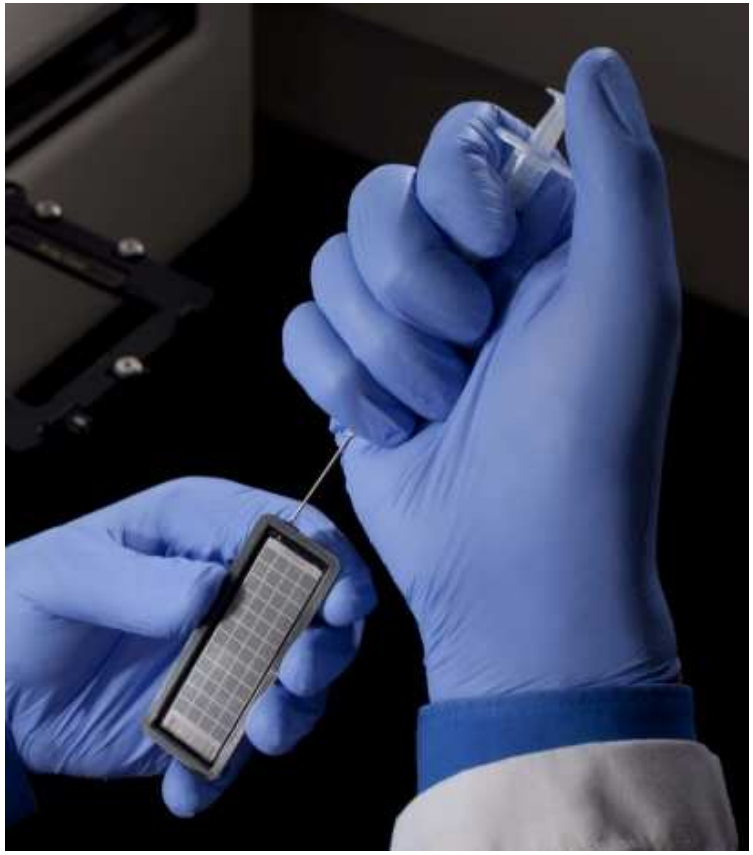
Seal OpenArray® Plate

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



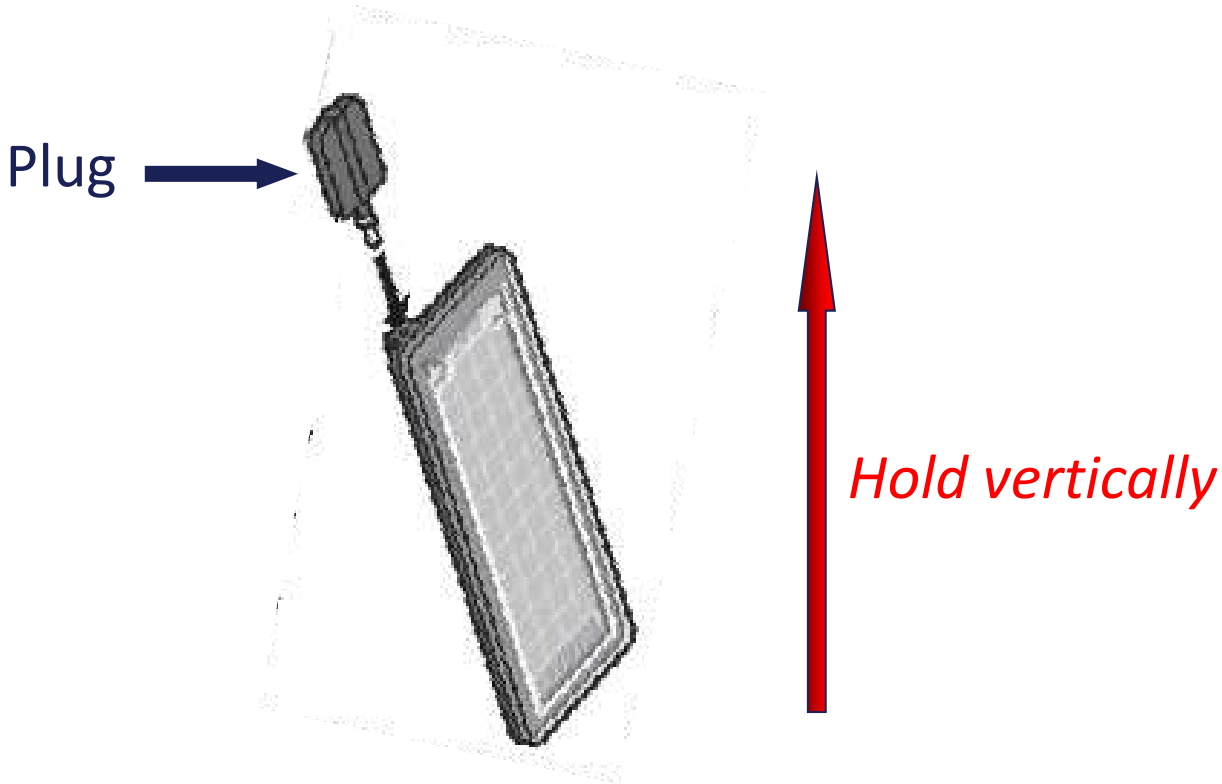
Immersion fluid

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



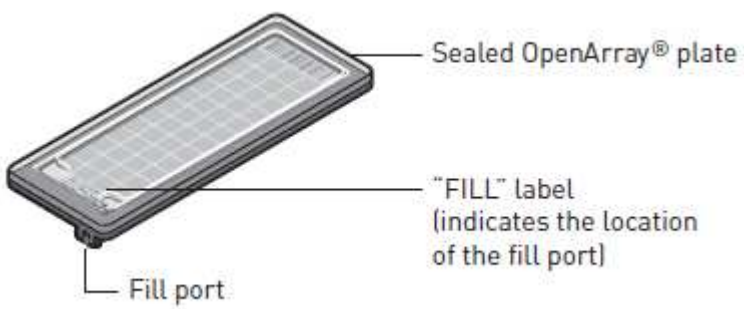
Plug the case

- 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

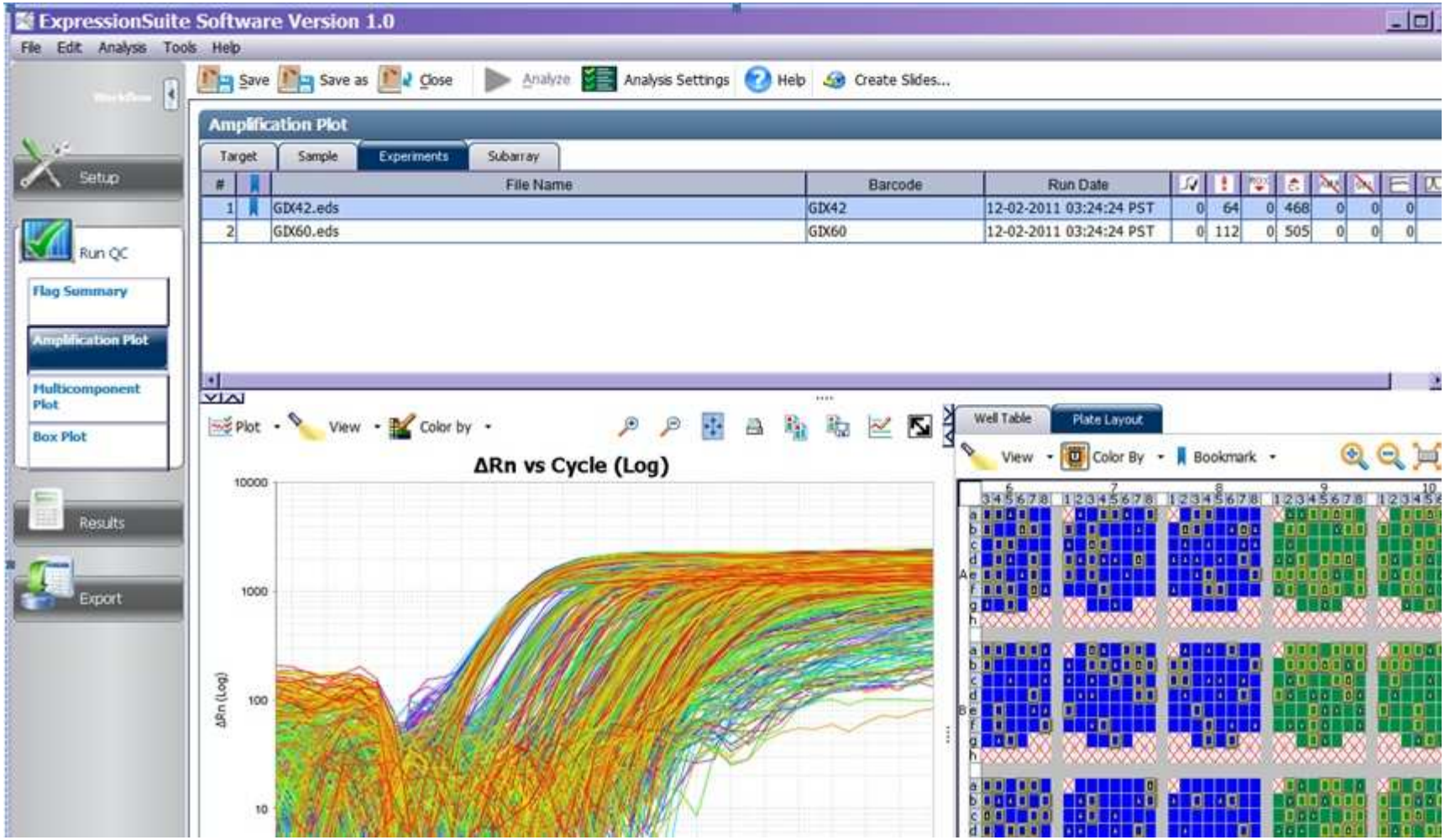


Load into the Instrument

- 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 lates after 2 hours of loading

MicroRNA Data Analyzed using ExpressionSuite™



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