Multiplex of Oncogene Target-Specific Panels

Introduction

The Access Array™ Target-Specific Panels provide efficient and effective primers for the analysis of BRCA1, BRCA2, TP53, EGFR and MET genes with one gene per Access Array integrated fluidic circuit (IFC). Combined with the Access Array System, they provide an easy and cost-effective workflow for targeted resequencing approaches to cancer research. Now the Access Array Target-Specific Panels can be multiplexed for the high-throughput analysis of the BRCA1, BRCA2 and TP53 or of the EGFR and MET panels in a single IFC. This multiplexing simplifies the workflow, saves valuable sample, and reduces experiment cost.

Workflow

Multiplex pooling	Access Array™ System	Barcoding	Library Preparation	Sequencing	Data Analysis

Results

FLUIDIGM TARGET-SPECIFIC PANEL SPECIFICATION	
% map to genome	> 90%
% map to target (map to genome)	> 95%
% coverage uniformity within 5 fold	> 85%

*Illumina Platforms only. The Ion Torrent™ PGM™ maps to the genome at >80%; indicative of the PGM.

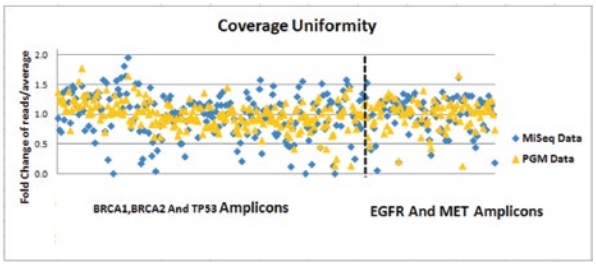
The results from the multiplex panel protocol validation have met Fluidigm's specifications for multiplex.

The following results may vary depend on customer's sample quality, sequencer's performance, and or other factors.

High Specificity and Uniformity

More than 90% of the reads mapped to genome using the Illumina(r) MiSeq Sequencer, more than 85% of the reads mapped to genome using the Ion Torrent (TM) Ion PGM(TM), with 99% of the reads mapped to the target region of exon from both sequencers. The results demonstrate the high specificity of the Access Array library.

More than 95% of the amplicons exhibit coverage within five fold of the average. This demonstrates the high uniformity of the Access Array library.



Conclusion

The multiplexing of the Oncogene Target-Specific Panels offers a simple workflow that targets all coding exons of either the BRCA1, BRCA2, and TP53 genes or the EGFR and MET genes. The high percentage of reads mapped to their target illustrates the specificity for targeted resequencing. This protocol combines the advantages of high sample throughput afforded by the Fluidigm® Access Array System with a multiplex strategy to screen for key oncogenes. The system provides a simple, fast, and accurate target-enrichment solution for the next-generation sequencing in oncogene studies.

Experimental Method

Required Reagets

- BRCA1/BRCA2/TP53 Target-Specific Panel (Fluidigm, PN 100-5451) OR
- EGFR/MET Target-Specific Panel (Fluidigm, PN 100-6060)
- FastStart High Fidelity PCR System, dNTPack (Roche, PN 04-738-292-001)
- PCR Certified Water (TEKnova, PN W3330)
- DNA Suspension Buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA) (TEKnova, PN T0221)
- Agencourt® AMPure® XP System Beckman Coulter, PN A63880)
- Agilent DNA 1000 Kit (Agilent, PN 5067-1504)
- Qubit® dsDNA HS Assay Kit (Life Technologies, PN Q32851)
- Genomic DNA at 50 ng/µL

For the MiSeq™ Sequencer:

- Access Array Barcode Library for Illumina Sequencers - 384 (Single direction) (Fluidigm, PN 100-4876)
- FL1 and FL2 Sequencing Primers (*Access Array System User Guide*, PN 100-7366)
- MiSeq Reagent Kit v2 (300 cycles) (Illumina, MS-102-200

For the Ion PGM™ Sequencer:

- Access Array Barcode Library for Ion Torrent PGM Sequencer - 96 (Bidirectional)
- (Fluidigm, PN 100-4911)
- Ion PGM Sequencing 200 Kit (Life Technologies, PN 4482006)
- Ion 316™ Chip Kit v2 (Life Technologies, PN 4483188)

Required Consumables

- Access Array 48.48 + Sample/Loading Kit 10 Chip Package (Fluidigm, PN AA - M10-48.48)
- Qubit® Assay Tubes (Life Technologies, PN Q32856, 500 tubes)
- PCR 8-well tube strip (VWR, PN 82006-606)
- 96-well PCR plates (VWR, 83009-672)
- P2–P1000 pipette tips (Rainin recommended)

Required Lab Equipment

- Access Array System (Fluidigm, PN AA - PKG-FC1)
- 2100 Electrophoresis Bioanalyzer (Agilent, PN G2939AA)
- DynaMag™-2 Magnet (Life Technologies, PN 123-21D)
- Qubit 2.0 Fluorometer (Life Technologies, PN Q32866)
- MiSeq Benchtop Sequencer (Illumina, SY-410-1003)

OR

- Ion Torrent Ion PGM System (Life Technologies, PN 4462921)

Preparing the Multiplexed Primer Stock

The following protocol gives the quantities needed to multiplex BRCA1, BRCA2, and TP53, or EGFR and MET. The quantities given here make 50 µL of multiplexed primer mix, which is enough to run ten Access Array IFCs. This mixture can be scaled up or down as needed.

NOTE: The multiple PCR primers from the same position of Oncogene Target-Specific Panels are evaluated by bioinformatic algorithms to ensure primer specificity and PCR efficiency.

1 Pipet 200 µL of DNA Suspension Buffer into each well of an 8-well strip or pour 1.5 mL of DNA Suspension Buffer in a reservoir.

2 Pipet 20 µL or 30 µL of DNA Suspension Buffer into 48 wells of a 96-well plate using an 8-channel pipette with the following map (see Figure 1) to make the same value for each well and achieve the same concentration for each primer.

Figure 1. Prefill Loading Map

BRCA1/BRCA2/TP53 prefill loading map

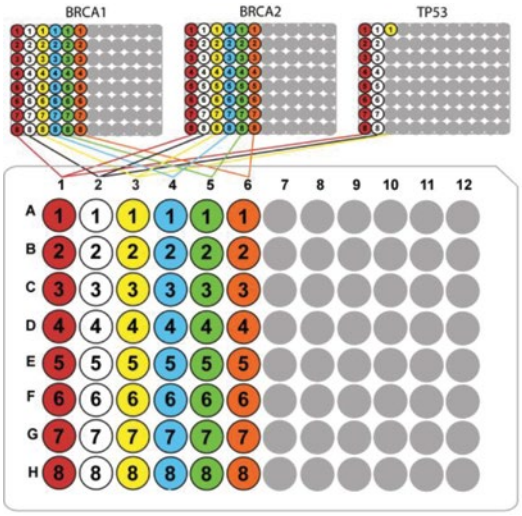
	1	2	3	4	5	6
A	20ul	20ul	20ul	30ul	30ul	30ul
B	20ul	20ul	30ul	30ul	30ul	30ul
C	20ul	20ul	30ul	30ul	30ul	30ul
D	20ul	20ul	30ul	30ul	30ul	30ul
E	20ul	20ul	30ul	30ul	30ul	30ul
F	20ul	20ul	30ul	30ul	30ul	30ul
G	20ul	20ul	30ul	30ul	30ul	30ul
H	20ul	20ul	30ul	30ul	30ul	30ul

EGFR/MET prefill loading map

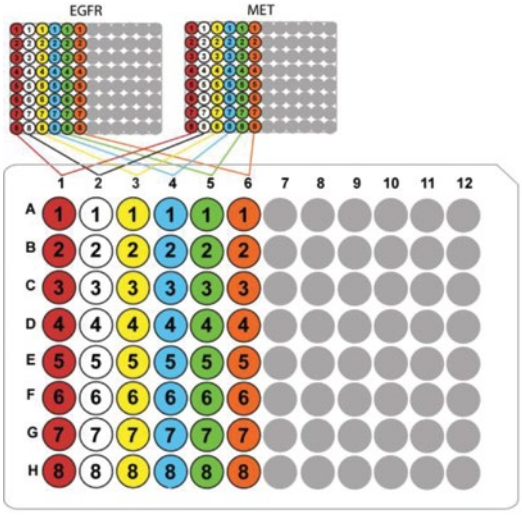
	1	2	3	4	5	6
A	30ul	30ul	30ul	30ul	30ul	30ul
B	30ul	30ul	30ul	30ul	30ul	30ul
C	30ul	30ul	30ul	30ul	30ul	30ul
D	30ul	30ul	30ul	30ul	30ul	30ul
E	30ul	30ul	30ul	30ul	30ul	30ul
F	30ul	30ul	30ul	30ul	30ul	30ul
G	30ul	30ul	30ul	30ul	30ul	30ul
H	30ul	30ul	30ul	30ul	30ul	30ul

3 Using an 8-channel pipette, combine 10 µL of primers from the same column of the Oncogene Target-Specific Panels to the designated column in the multiplexed stock primer plate (see Figure 2).

Figure 2. Multiplexed Primer Stock Map



BRCA1/BRCA2/TP53 Multiplexed Primer Stock



EGFR/MET Multiplexed Primer Stock

Preparing 20X Primer Solutions

(!) **IMPORTANT:** The concentration of each primer in the 20X mix is 1 µM. In the reaction, the final primer concentration is 50 nM.

1 Pipet 20 µL of Access Array loading reagent into each well of an 8-well strip.

2 Pipet 2.5 µL of Access Array loading reagent into each of the 48 wells of the multiplexed primer stock prepared in the previous section, "Preparing the Multiplexed Primer Stock," to make 20X primer solutions using an 8-channel pipette.

3 Seal the 20X Primer Solution Plate with an adhesive seal, vortex it for a minimum of 20 seconds, and centrifuge for 30 seconds to spin down all components.

4 The solutions are ready to be loaded into the Primer Inlets of a 48.48 Access Array IFC.

OR

The solutions can be stored at 4 °C for short-term use. For intermittent use, store at –20 °C for up to six months.

Preparing the Sample Pre-Mix and Sample Mix

1 Combine the components in the following table to make the Sample Pre-Mix in a 1.5-mL microcentrifuge tube (scale up appropriately for multiple runs):

Component	Vol. Per Reaction (µL)	Vol. for 60 Reactions (µL)	Final Conc.
10X FastStart High Fidelity Reaction Buffer without $MgCl_2$	0.50	30.0	1X
25 mM $MgCl_2$	0.90	54.0	4.5 mM
DMSO	0.25	15.0	5%
10mM PRC Grade Nucleotide Mix	0.10	6.0	200 µM ea
5 U/µL FastStart High Fidelity Enzyme Blend	0.05	3.0	0.05 U/µL
20X Access Array Loading Reagent	0.25	15.0	1X
PCR Certified Water	1.95	117.0	
Total	**4.00**	**240.0**	

2 Vortex the Sample Pre-Mix for a minimum of 20 seconds, and centrifuge for 30 seconds to spin down all components.

3 Pipet 28 µL of Sample Pre-Mix into each well of an 8-well strip.

4 Pipet 4 µL of Sample Pre-Mix into 48 wells of a 96-well plate using an 8-channel pipette.

5 Add individual sample to combine the components listed below to prepare the sample mix solutions:

Component	Volume per Reaction (µL)
Sample pre-Mix	4.0
Genomic DNA (50 ng/µL)	1.0
Total	5.0

6 Seal the sample solution plate with an adhesive seal, vortex it for a minimum of 20 seconds, and centrifuge for 30 seconds to spin down all components.

7 The final sample mix solutions are ready to be loaded into the Sample Inlets of the Access Array IFC.

Priming the Access Array IFC

NOTE: For more information on the Access Array System, refer to the Access Array System User Guide (PN 100-7366).

1 Inject control line fluid into each accumulator on the IFC.

2 Add 500 µL of 1X Access Array Harvest Solution into the H1-H4 wells on the IFC.

3 Remove and discard the protective blue film from the bottom of the Access Array IFC.

4 Press **Eject** to move the tray out of the IFC Controller AX.

5 Place the IFC onto the tray by aligning the notched corner of the IFC to the A1 mark.

6 Press **Load Chip** to register the barcode of the IFC and activate the script selection.

7 Select **Prime (151x)** and **Run Script** to prime the IFC.

8 When the script is complete, press **Eject** to remove the IFC.

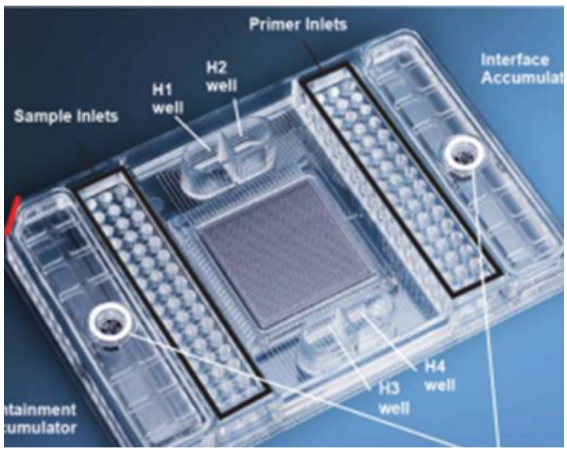
9 The priming step takes about 10 minutes.

NOTE: We recommend using an 8-channel pipette when preparing samples, primers and working with the Access Array IFC.

CAUTION: While pipetting, do not go past the first stop on the pipette. Doing so may introduce air bubbles into the inlets.

Loading the Access Array IFC

Figure 3. 48.48 Access Array IFC



1 Pipet 4 µL of 20X Primer Solution into each of the Primer Inlets on the IFC.

2 Pipet 4 µL of Sample Mix Solution into each of the Sample Inlets on the IFC.

3 Press Eject to move the tray out of the IFC Controller AX.

4 Place the IFC onto the tray by aligning the notched corner of the IFC to the A1 mark.

5 Select **Load Chip** to register the barcode of the IFC and activate the script selection.

6 Select **Load Mix (151x)** and **Run Script.**

7 Once the script is complete, press Eject to remove the IFC.

8 The Load Mix step takes about 60 minutes.

Thermal Cycling the 48.48 Access Array IFC

1 Place the 48.48 Access Array IFC onto the FC1™ Cycler.

2 Start PCR by selecting the protocol **AA 48X48 Standard v1.**

Harvesting the 48.48 Access Array IFC

1 When PCR has finished, move the 48.48 Access Array IFC into the Post-PCR lab for harvesting.

2 Remove the remaining 1X Access Array Harvest Reagent from the H1–H4 wells.

3 Pipet 600 µL of fresh 1X Access Array Harvest Reagent into the H1–H4 wells.

4 Pipet 15 µL of 1X Access Array Harvest Reagent into each well of an 8-well strip.

5 Pipet 2 µL of 1X Access Array Harvest Reagent into each of the Sample Inlets on the IFC using an 8- channel pipette.

6 Load the IFC into the Post-PCR IFC Controller AX, located in the Post-PCR lab.

7 Press **Eject** to move the tray out of the IFC Controller AX.

8 Place the IFC onto the tray by aligning the notched corner of the IFC to the A1 mark.

9 Press **Load Chip** to register the barcode of the IFC and activate the script selection.

10 Select **Harvest (151X)** and Run Script.

11 When the script is complete, press **Eject** to remove the IFC.

12 Label a 96-well plate with the 48.48 Access Array IFC barcode.

13 Carefully transfer 10 µL of the harvested PCR products from each of the Sample Inlets into the 96-well PCR plate.

Preparing a 100-Fold Dilution of the Harvested PCR Products

1 Label a 96-well plate "1:100 Diluted Sample."

2 Pipet 198 µL of PCR Certified Water into each of the 48 wells to prepare the 1:100 diluted sample.

3 Add 2 µL of each harvested PCR product from the harvesting step to each well of the 1:100 Diluted Sample plate.

4 Seal the 1:100 Diluted Sample plate with an adhesive seal, vortex it for a minimum of 20 seconds, and centrifuge for 30 seconds to spin down all components.

Attaching Sequence Tags and Sample Barcodes

(!) **NOTE:** The library prepared from the Access Array System can be barcoded either for Illumina or Ion Torrent platforms.

1 Combine the components in the following table to make the Sample Pre-Mix in a 1.5-mL microcentrifuge tube:

Component	Vol. Per Reaction (µL)	Vol. for 60 Reactions (µL)	Final Conc.
10X FastStart High Fidelity Reaction Buffer **without** $MgCl_2$	2.0	120.0	1X
25 mM $MgCl_2$	3.6	216.0	4.5mM
DMSO	1.0	60.0	5%
10mM PRC Grade Nucleotide Mix	0.4	24.0	200 µM ea
5 U/µL FastStart High Fidelity Enzyme Blend	0.2	12.0	200 U/µL
PCR Certified Water	7.8	468.0	
Total	**15.0**	**900.0**	

2 Vortex the Sample Pre-Mix for a minimum of 20 seconds, and centrifuge for 30 seconds to spin down all components.

3 Pipet 15 µL of Sample Pre-Mix into 48 wells of a 96-well plate using an 8-channel pipette.

4 Add the individual 100-fold diluted sample to combine the components listed below:

Component	Volume per Reaction (µL)
Sample Pre-Mix	15.0
Access Array Barcode Library for Illumina Sequencers - 384 - Single Direction [Or Access Array Barcode Library for Ion Torrent™ PGM Sequencer - 96 (Bidirectional)]	4.0
1:100 DILUTED SAMPLE	1.0
Total	20.0

5 Seal the Sample Mix plate with an adhesive seal, vortex it for a minimum of 20 seconds, and centrifuge for 30 seconds to spin down all components.

6 Label this plate "Barcoded Sample."

Thermal Cycling to Add Sequence Tags and Barcode

1 Place the Barcoded Sample plate on the PCR thermal cycler and run the following PCR protocol:

PCR Stages	Number of Cycles
95º C 10 min	1
95º C 15 min 60º C 30 min 72º C 1 min	15
72º C 3 min	1

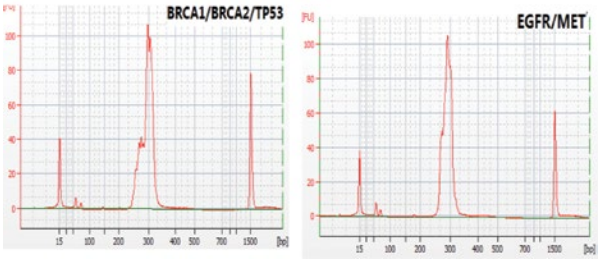
2 Remove the plate to continue to the next step.

Checking PCR Products on the Agilent 2100 Bioanalyzer

1 Use the Agilent DNA 1000 chip from the Agilent DNA 1000 Kit to check 1 µL of PCR product from each of the PCR reactions described above. Follow the *Agilent DNA 1000 Kit Quick Start Guide* (PN G2938-90015) for details.

2 Check the results of the chip to determine if the PCR product has 294 bp ± 5% for BRCA1, BRCA2 and TP53 or 299 bp ± 5% for EGFR and MET; a smear may be visible.

3 The following electropherograms are examples of the BRCA1, BRCA2, and TP53 product and the EGFR and MET product:



4 Proceed to the next step for sequencing library preparation.

OR

Store the PCR products at –20ºC for a long term use.

Pooling Products

1 Pool all the barcoded samples in equal volume to create a harvest sample pool as a single library.
OR
Pool the selected barcoded samples that need to be sequenced.

Component	Volume (µL)	Total Volume (µL)
Pooled barcoded samples (48 RXN)	5.0	240.0

Purification of Harvested PCR Products

1 Remove AMPure XP beads from refrigerator and warm up at room temperature for 30 minutes.
   a Prepare a fresh 70% ethanol solution:
   b To a 15-mL tube, add 3 mL of PCR Certified Water and 7 mL of 100% ethanol.

2 Vortex for 5 seconds.

3 Vortex AMPure XP beads for 10 seconds to resuspend. The bead solution should appear homogeneous and consistent in color.

4 Pipet the harvest sample pool, DNA Suspension Buffer, and AMPure XP beads into a 1.5-mL microcentrifuge tube according to the table below:

Component	Total Volume (µL)
Harvest sample pool	24.0
DNA Susppension Buffer	48.0
Total	**72.0**

5 Vortex the tube and incubate at room temperature for 10 minutes.

6 Place the tube onto a magnetic separator and allow it to sit for 1 minute.

7 Carefully pipet out the supernatant without disturbing the beads (remove as much liquid as possible).

8 Add 180 µL of 70% ethanol and vortex for 10 seconds.

9 Place the tube onto a magnetic separator and allow it to set for 1 minute.

10 Carefully pipet out the supernatant without disturbing the beads.

11 Add 180 µL of 70% ethanol and vortex for 10 seconds.

12 Place the tube onto a magnetic separator and allow it to set for 1 minute.

13 Carefully pipet out the supernatant without disturbing the beads.

14 Allow the beads to air dry for approximately 10 minutes by leaving the tube on the bench. Make sure the tube is completely dry before proceeding.

15 Add 80 µL of DNA Suspension Buffer to the tube and vortex for 5 seconds.

16 Place the tube onto a magnetic separator and allow it to set for one minute.

17 Carefully transfer the supernatant to a 1.5-mL microcentrifuge tube, labeled "Purified Sample."

Quantification by Qubit 2.0 Fluorometer

NOTE: For more information about operating the Qubit 2.0 Fluorometer, refer to the *Qubit 2.0 Fluorometer User Manual* (Life Technologies, PN MAN0003231).

1 Prepare working solution by diluting the Qubit reagent 1:200 in Qubit buffer:

Component	Volume (µL)
Qubit Reagent	4.0
Qubit Buffer	796.0
Total	800.0

2 Prepare the standard sample tubes according to the table below:

Component	Volume (µL)
Working Stock	190.0
Standard Sample 1 and 2	10.0
Total	200.0

3 Prepare the sample tube according to the table below:

Component	Volume (µL)
Working Stock	198.0
Purified Sample	2.0
Total	200.0

4 Vortex all tubes for 2–3 seconds.

5 Insert the tubes in the Qubit 2.0 Fluorometer and take readings.

6 Calculate the sample concentration X (ng/µL) by the Qubit 2.0 Fluorometer.

7 Calculate the sample concentration Y (nM) by the following formula:

$Y = X / (660 * (\text{Avg. length of amplicons})) * 10^6$

- Average length of amplicons BRCA1, BRCA2, and TP53 is 294 bp.
- Average length of amplicons EGFR and MET is 299 bp.
- Fluidigm® data indicates X = 4.1 ng/µL and Y = 21.0 nM. Your data may vary depending on the quality of the input sample.

NOTE: For Ion Torrent sequencing run with the library prepared in the previous steps, refer to the *Ion PGM Template OT2 200 Kit User Guide* (Life Technologies, PN MAN0007220) and the *Ion PGM Sequencing 200 Kit v2 User Guide* (Life Technologies, PN MAN0007273).

Preparation of 7 pM Denatured Library for MiSeq® Run

NOTE: For more information on MiSeq operation, refer to the *MiSeq System User Guide* (PN 15027617).

1 Prepare 10 mM Tris-HCl, pH 8.5 with 0.1% Tween® 20:

Component	Volume (µL)
1 M Tris-HCl, pH8.5	0.4
Tween 20	0.04
PCR Certified Water	39.56
Total	40.0

2 Prepare 500 µL of 0.2N NaOH by combining the following components in a 1.5-mL microcentrifuge tube. Invert the tube several times to mix:

Component	Volume (µL)
10 N NaOH	10.0
PCR Certified Water	490.0
Total	40.0

3 Combine the following components in a 1.5-mL microcentrifuge tube to prepare the 2 nM library:

Component	Volume (µL)
Library	5.0
10 mM Tris-HCl with 0.1% Tween 20	(5 * Y/2) – 5

*Y is the sample concentration calculated from Quantification step.

4 Combine the following components in a 1.5-mL microcentrifuge tube to prepare the 2 nM library:

Component	Volume (µL)
2 nM Library	10.0
0.2 N NaOH	10.0

5 Vortex briefly to mix the sample dilution, then centrifuge the sample solution for 1 minute.

6 Incubate for 5 minutes at room temperature to denature the DNA into single strand.

7 Add 980 µL of prechilled HT1 into the tube to make 20 pM denatured library.8.

8 Combine the following components in a 1.5-mL microcentrifuge tube to make 7 pM denatured library.

Component	Volume (µL)
20 pM denatured Library	350.0
HT1	650.0
Total	1000.0

9 Invert the tube several times to mix, then pulse centrifuge the DNA solution.

10 Place the denatured diluted DNA on ice until you are ready to load your sample onto the MiSeq reagent cartridge.

Performing a MiSeq Run

NOTE: For information on ordering and preparing Fluidigm sequencing primers FL1 and FL2 for the MiSeq Sequencer, refer to the Access Array System User Guide (PN 100-7366).

NOTE: For guidance on using Phix control in a sequencing run, please contact Fluidigm Technical Support.

1 A sample sheet template is available for download from: support.illumina.com/sequencing/downloads.ilmn, or a previous sample sheet can be used as the template for modification.

2 Change the following parameters: Investigator, Project Name, Experiment Name, and Date.

[Header]						
IEMFileVersion	4					
Investigator Name	Name					
Project Name	MultiplexOncogenePanel					
Experiment Name	OncogeneMultiplex					
Date	6/1/2013					
Workflow	Resequencing					
Application	Resequencing					
Assay	TruSeq Amplicon					
Description						
Chemistry	Default					
[Reads]						
151						
151						
[Data]						
Sample_ID	Sample_Na	Sample_P	Sample_V	Sample_P	index	I7_Index_
M1_S1					GTATCGTCG	FLD0001
M1_S2					GTGTATGCG	FLD0002

3 Change Workflow to “Resequencing.”

4 Change Application to “Resequencing.”

5 Change Assay to “TruSeq Amplicon.”

6 In the Reads section, change the values to “151.”

7 Create a Settings section after the Reads section, then add three lines for Fluidigm custom sequencing primers:

Settings	
CustomRead1PrimerMix	C1
CustomRead1PrimerMix	C2
CustomRead1PrimerMix	C3

8 Change the Sample ID to the sample name.

9 Use the sequence of the Fluidigm barcode as Index.

10 Use the Barcode name “I7 Index.”

11 Save the sample sheet using the barcode number of the reagent cartridge with a .csv extension.

12 Copy the sample sheet to the instrument computer under the folder: Illumina\MiSeq Control Software\SampleSheets.

13 Remove the MiSeq Reagent Kit (Box 1) from the –20 °C freezer, and place it in a water bath for 1 hour. Do not allow the water to exceed the maximum water line printed on the reagent cartridge.

14 Label a 1.5-mL PCR tube with “Sequence Primer.”

15 Add 1386 µL of chilled HT1 and 14 µL of FL1 primers at 50 µM per primer.

16 Vortex for 5 seconds and place the tube on ice.

17 Label a 1.5-mL PCR tube with “Index Primer.”

18 Add 693 µL of chilled HT1 and 7 µL of FL2 primers at 50 µM.

19 Vortex for 5 seconds and place the tube on ice.

20 Pierce the Sample Load Port as indicated on the reagent cartridge using a clean 1-mL pipette tip.

21 Pipet 600 µL of 7 pM sample library into the sample port.

22 Pierce Ports 18, 19, and 20 using a clean 1-mL pipette tip.

23 Pipet 680 µL of the Sequence Primer into each of Ports 18 and 20.

24 Pipet 680 µL of the Index Primer into Port 19.

25 Tap the cartridge 2–3 times.

26 Rinse the flow cell with the distilled water and dry it with lint-free tissues moistened with ethanol or isopropanol and make sure there are no water drops on the surface of the flow cell.

27 Follow the instructions on the sequencer to start the run.

28 Perform the Post-Run Wash after completing the sequencing run.

Data Analysis

- MiSeq reporter or BaseSpace can be used to analyze the data.
- Ion Reporter Software can be used to analyze the data.
- The third-party software can be selected to meet different needs.
- Fluidigm uses an in-house pipeline to analyze the data from FASTQ files generated from MiSeq Reporter or BaseSpace.

References

1 *Access Array System User Guide*(Fluidigm, PN 100-7366)

2 *Agilent DNA 1000 Kit Guide* (Agilent, PN G2938-90014)

3 *MiSeq System User Guide* (Illumina, PN 15027617)

4 *Agencourt AMPure XP Protocol* (Agencourt, PN 000387v001)

5 *Qubit 2.0 Fluorometer User Manual* (Life Technologies, MAN0003231)

For More Information

To find out more about the information in this or any other Fluidigm Technical Note, contact techsupport@fluidigm.com.

Disclaimer

Corporate headquarters
7000 Shoreline Court, Suite 100
South San Francisco, CA 94080 USA
Toll-free: 866 359 4354 | Fax: 650 871 7152
fluidigm.com

Sales
North America | +1 650 266 6170 | info-us@fluidigm.com
Europe/EMEA | +33 (0)1 60 92 42 40 | info-europe@fluidigm.com
Japan | +81 (0)3 3662 2150 | info-japan@fluidigm.com
China (excluding Hong Kong) | +86 (0)21 3255 8368 | info-china@fluidigm.com
Asia | +1 650 266 6000 | info-asia@fluidigm.com
Latin America | +1 650 266 6000 | info-latinamerica@fluidigm.com

PN 100-2113 B1