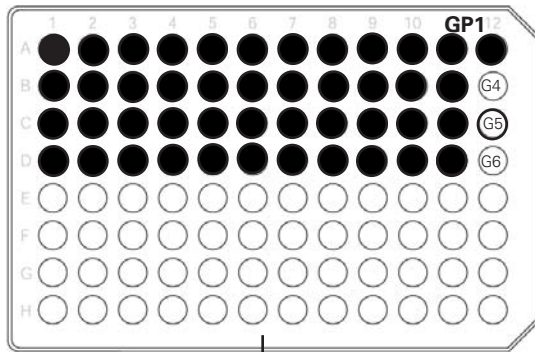


Quick Reference Card

DMET Plus Protocol Stage 1 – mPCR

In the Pre-Amp Lab

1. Prepare Genomic Plate 1 (GP1; 60 ng/μL).



● = 17 μL each genomic DNA sample
(at a concentration of 60 ng/μL)

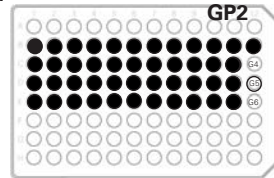
⊗ = 17 μL each gDNA control

NOTE: Genomic controls may change over time. For the HAPMAP identifier of the current control, please see manual.

2. Prepare Genomic Plate (GP2; 10 ng/μL).

To each well:

- Add 10 μL 1X TE Buffer.
- Transfer 2 μL each sample/control from plate GP1.
- Seal, vortex and spin down.



3. Prepare mPCR Master Mix (mix 5X with P1000 set to 900 μL; do not vortex)

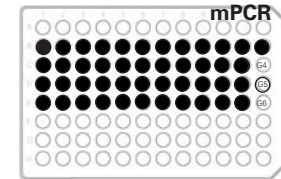
mPCR MASTER MIX			
Reagent	Per Sample	48 Samples (> 20% extra)	Lot Number
QIAGEN Multiplex PCR Master Mix	25 μL	1500 μL	
mPCR Primer Mix (3 μM)	5 μL	300 μL	
5X Q-Solution	5 μL	300 μL	
RNase-free Water	10 μL	600 μL	
Total Volume	45 μL	2700 μL	—

PCR Dilution Buffer	—	—	
TE Buffer (1X)	—	—	

4. Prepare Plate mPCR.

To each well:

- Add 45 μL mPCR Master Mix.
- Transfer 5 μL each sample/control from plate GP2.
- Seal, vortex and spin down.



5. Plate mPCR onto thermal cycler.

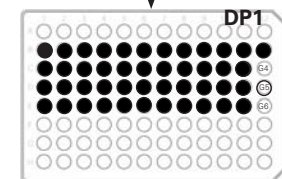
DMET Plus mPCR		
Temp	Time	Cycles
94 °C	15 min	—
95 °C	30 sec	35 Cycles
60 °C	90 sec	
72 °C	45 sec	
72 °C	3 min	—
4 °C	Hold	—

In the mPCR Staging Area

6. Prepare Dilution Plate 1 (DP1).

To each well:

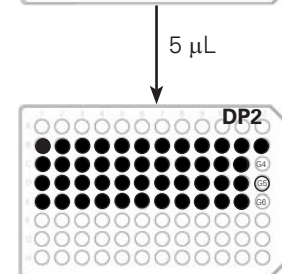
- Add 153 μL PCR Dilution Buffer.
- Transfer 5 μL each sample/control from plate mPCR.
- Mix slowly 10X with P100 set to 80 μL. Do not blow out pipet tips.



7. Prepare Dilution Plate 2 (DP2).

To each well:

- Add 153 μL PCR Dilution Buffer.
- Transfer 5 μL each sample/control from plate DP1.
- Mix slowly 10X with P100 set to 80 μL. Do not blow out pipet tips.
- Seal plate DP2; discard plate DP1.



Quick Reference Card

DMET Plus Protocol Stage 2 – Anneal

In the Pre-Amp Lab

1. Prepare the Anneal Master Mix (mix 5X with P1000 set to 900 μL ; do not vortex).

Anneal MASTER MIX			
Reagent	Per Sample	48 Samples (> 25 % extra)	Lot Number
Pre-Amp Water	16.6 μL	996 μL	
Buffer A	5 μL	300 μL	
Enzyme A	0.0625 μL	3.8 μL	
Total Volume	21.7 μL	1299.8 μL	—

IMPORTANT Enzyme A:

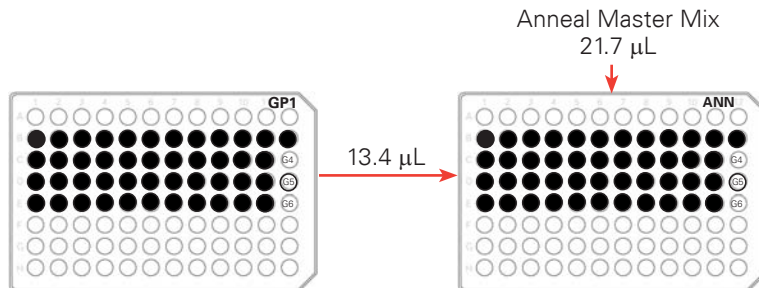
- Thaw on bench top until defrosted only; then place on ice. Do NOT vortex.
- Extremely temperature sensitive. Keep master mix on ice to avoid denaturing.

2. Load the Anneal Plate (ANN).

To each well:

- Add 21.7 μL Anneal Master Mix to each sample/control.
- Transfer 13.4 μL each sample from GP1 to ANN.

Total volume each well: 35.1 μL

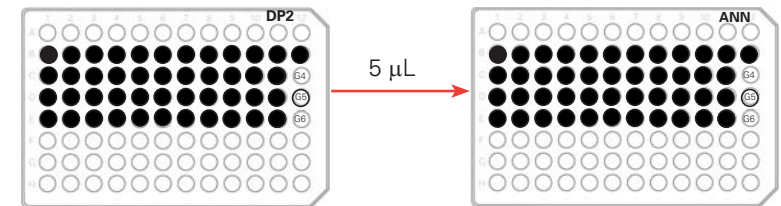


3. Seal and transfer plate ANN to the mPCR Staging Area.

In the mPCR Staging Area

4. Add 5 μL diluted mPCR product (plate DP2) to plate ANN.

Total volume each well: 40.1 μL



5. Seal and transfer plate ANN to the Pre-Amp Lab.

In the Pre-Amp Lab

6. Vortex and spin plate ANN. Then place onto thermal cycler and start DMET Plus Anneal program.

7. Add DMET MIP Panel to plate ANN. DMET MIP Panel Lot # _____

At the end of the first 95 deg C hold:

- Remove plate and cool in aluminum block on ice for 2 min.
- Aliquot to strip tubes, 25 μL each tube.
- Add 5 μL DMET MIP Panel to each reaction.
- Seal plate, vortex, spin down.
- Put plate back on thermal cycler and resume the program.
- Incubate plate for 16 to 18 hr. Do not exceed 18 hr.

DMET Plus Anneal	
Temp	Time
20 °C	4 min
95 °C	5 min
95 °C	5 min
58 °C	Hold

Add 5 μL DMET MIP Panel to each reaction

Total volume per well: 45.1 μL

Quick Reference Card

DMET Plus Protocol

Stage 3 – Gap Fill through Amplification

In the Pre-Amp Lab

1. Add Gap Fill Mix to Anneal Plate.

- Add 2.5 μL Gap Fill Mix to each well.
- Seal, vortex, spin.

2. Transfer aliquots from Anneal to Assay Plate.

3. Assay plate to thermal cycler – run DMET Plus Assay.

4. After first 11 min, chill plate for 2 min, spin, add dNTP Mix.

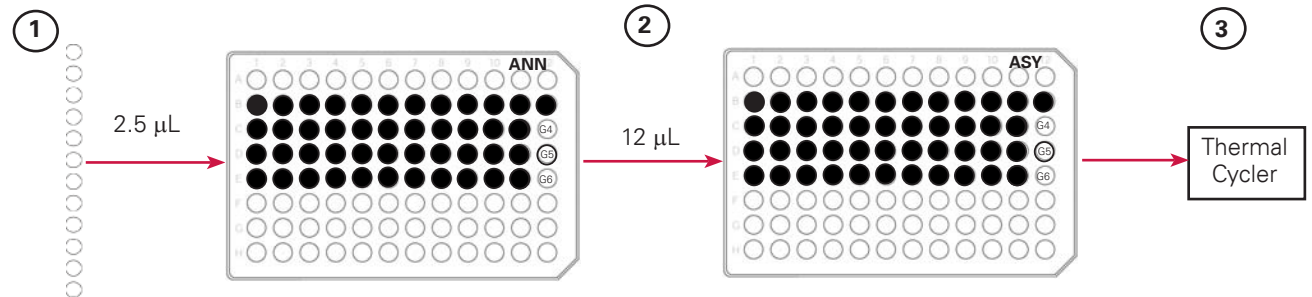
Seal, vortex spin. Resume program.

5. When thermal cycler reaches 37 $^{\circ}\text{C}$, chill plate for 2 min, spin, add Exo Mix.

Seal, vortex spin. Resume program.

6. When thermal cycler reaches 60 $^{\circ}\text{C}$, chill plate for 2 min, spin, add Universal Amp Mix.

Seal, vortex spin. Resume program.



Gap Fill Mix

- Spin Gap Fill Mixes 1 and 2.
- To an Eppendorf tube:
 - Slowly add 190 μL Gap Fill Mix 2.
 - Slowly add 10 μL Gap Fill Mix 1.
 - Mix 15X with P200 set to 150 μL .

Aliquot to strip tubes, 14 μL each tube.

Lot numbers:
 Gap Fill Mix 1: _____
 Gap Fill Mix 2: _____

dNTP Mix

- Vortex and spin.
- Aliquot to strip tubes, 25 μL each tube.

Lot number: _____

Exo Mix

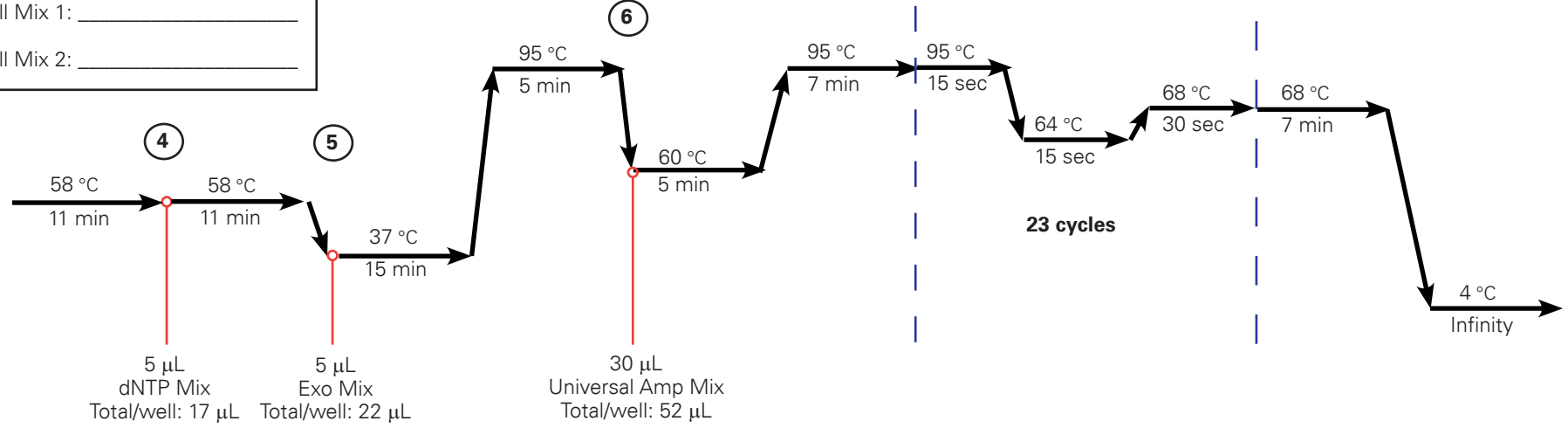
- Spin (no vortex).
- Aliquot to strip tubes, 25 μL each tube.

Lot number: _____

Universal Amp Mix

- Vortex and spin Universal Amp Mix tube (UAM).
- Spin Cleavage Enzyme and TITANIUM Taq.
- Add 25 μL Cleavage Enzyme to UAM tube.
- Add 70 μL Taq Polymerase to UAM tube.
- Mix 10X with P1000 set to 900 μL .
- Pour into reagent reservoir.

Lot numbers:
 Universal Amp Mix: _____
 Cleavage Enzyme: _____
 TITANIUM Taq: _____



Quick Reference Card

DMET Plus Protocol

Stage 4 – PCR Cleanup and First QC Gel

In the Post-Amp Lab

1. Add PCR Cleanup Mix

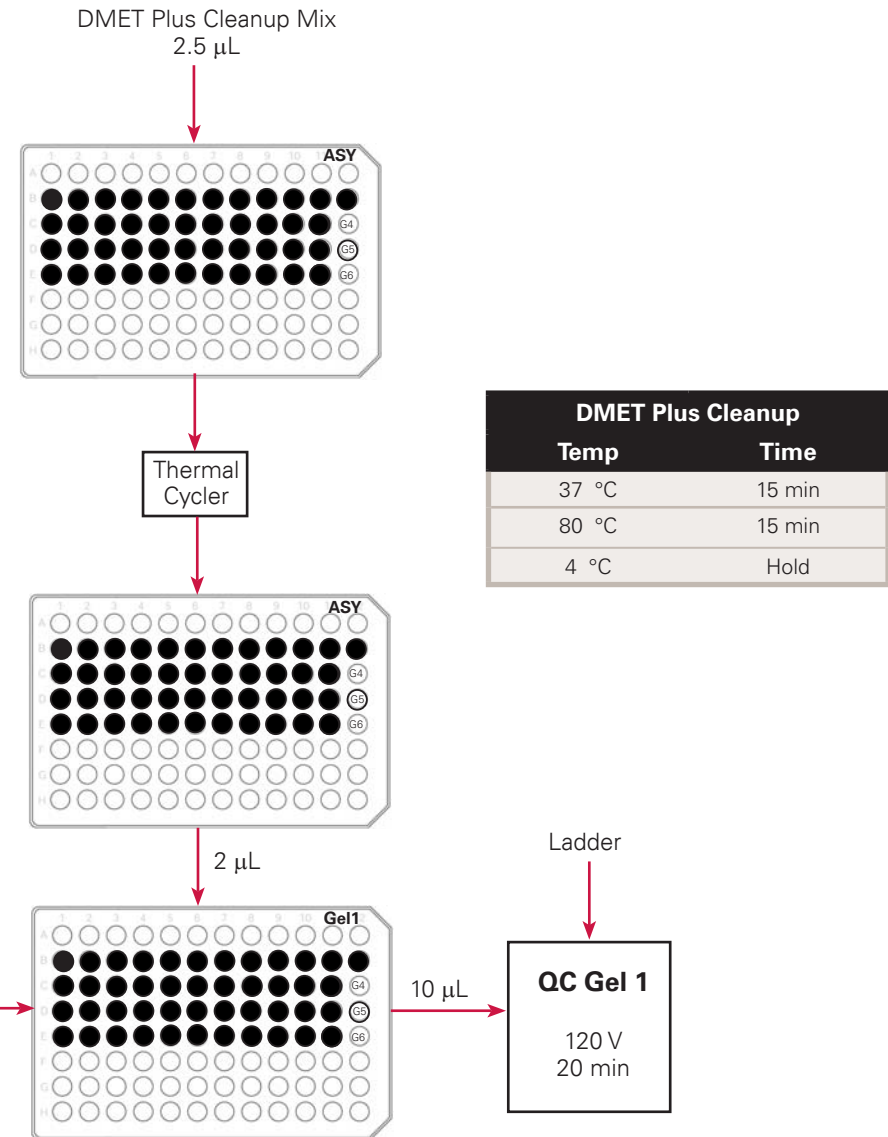
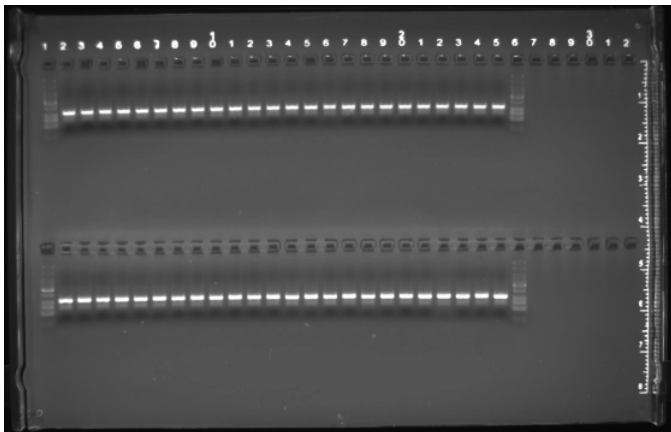
- Spin down.
- Aliquot to strip tubes, 15 μ L each tube.
- Add 2.5 μ L to each reaction on ASY plate (total volume 54.5 μ L).
- Seal, vortex, and spin.
- Plate to thermal cycler; run DMET Plus Cleanup.

PCR Cleanup Mix Lot Number: _____

2. QC Gel 1

- Remove plate from thermal cycler and spin.
- Aliquot 8 μ L 1X TE Buffer to the gel plate.
- Add 2 μ L of 2X Loading Buffer.
- Transfer 2 μ L each reaction from the ASY plate to the gel plate.
- Seal, vortex, spin the gel plate.
- Load 10 μ L each reaction and the ladder onto a 3% agarose gel.
- Run the gel at 120 V for 20 min.

Example of good QC Gel 1



To each well used:
 • 8 μ L 1X TE Buffer
 • 2 μ L 2X Loading Buffer

Quick Reference Card

DMET Plus Protocol

Stage 5 – Fragmentation and Second QC Gel

In the Post-Amp Lab

1. Transfer Reactions to Frag/Label Plate

A. Transfer 25 μ L each reaction from the ASY to the Frag/Label plate.

2. Prepare the Fragmentation Master Mix

A. To a 1.5 mL Eppendorf tube add:

- 536 μ L Post-Amp Water
- 60 μ L Fragmentation Buffer

B. Cool on ice for 5 min.

C. Add 4.1 μ L Fragmentation Reagent.

D. Vortex, spin and place on ice.

E. Aliquot to strip tubes, 45 μ L each tube.

3. Add the Fragmentation Master Mix to the Frag/Label Plate

A. Add 10 μ L Fragmentation Master Mix to each reaction (total volume 35 μ L).

B. Seal, vortex, spin.

C. Plate to thermal cycler; run DMET Plus Frag.

4. QC Gel 2

A. Remove plate from thermal cycler and spin.

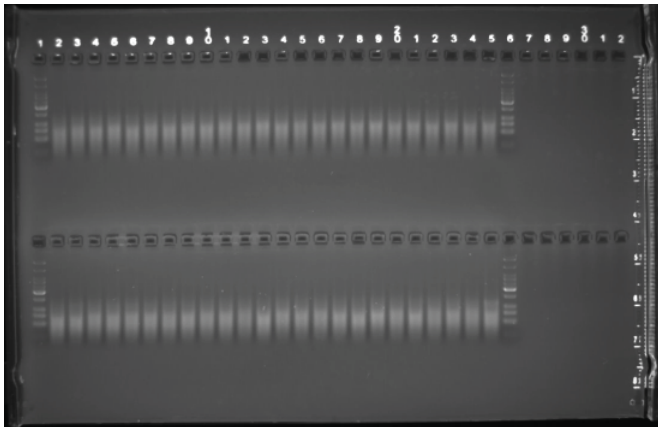
B. Transfer 10 μ L each reaction from the Frag/Label plate to the gel plate.

C. Add 2 μ L 2X Loading Buffer.

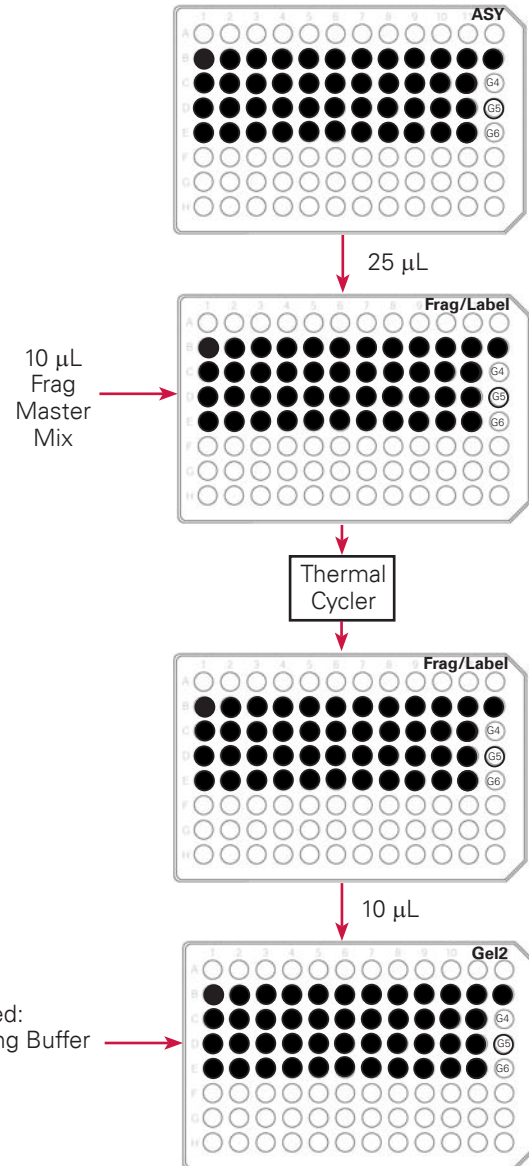
D. Seal, vortex, spin the gel plate.

E. Load 10 μ L each reaction and the ladder onto a 3% agarose gel.

F. Run the gel at 120 V for 24 min.



Example of good QC Gel 2



Reagent Lot Numbers:

Post-Amp Water: _____

Frag Buffer: _____

Frag Reagent: _____

DMET Plus Frag	
Temp	Time
37 °C	15 min
95 °C	15 min
4 °C	Hold

Quick Reference Card

DMET Plus Protocol Stage 6 – Labeling

In the Post-Amp Lab

1. Prepare the Labeling Master Mix

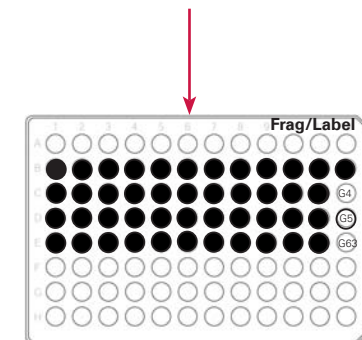
- Prepare in 1.5 mL Eppendorf tube.
- Vortex, spin and place on ice.
- Aliquot to strip tubes, 45 μL each tube.

LABELING MASTER MIX			
Reagent	Per Sample	48 Samples (> 20 % extra)	Lot Number
Post-Amp Water	0.4 μL	24 μL	
5X TdT Buffer	7 μL	420 μL	
DNA Labeling Reagent	0.9 μL	54 μL	
TdT Enzyme	1.7 μL	102 μL	
Total Volume	10 μL	600 μL	—

2. Add the Labeling Master Mix to the Frag/Label Plate

- Add 10 μL Labeling Master Mix to each reaction (total volume 35 μL).
- Seal, vortex, spin.
- Plate to thermal cycler; run DMET Plus Label.

10 μL Labeling
Master Mix



Thermal
Cycler

DMET Plus Label	
Temp	Time
37 °C	60 min
95 °C	15 min
4 °C	Hold

Quick Reference Card

DMET Plus Protocol Stage 7 – Hybridization

In the Post-Amp Lab

1. Preheat the Hyb Oven

- A. Preheat to 49 deg C with rotation on (35 rpm).

2. Prepare the Arrays

- A. Unwrap and warm to room temperature.
- B. Mark with sample designation.
- C. Insert 200 μ L pipet tip in upper right septum.

3. Prepare the Hyb Master Mix and Aliquot to Hyb Plate

- A. To the Hybridization Solution tube, add 50 μ L Oligo Control Reagent.
- B. Mix by inverting tube 10X.
- C. Pour into a reagent reservoir and place on ice.
- D. Wet pipet tips by aspirating/dispersing 3X.
- E. Aliquot 92 μ L Hyb Master Mix to the Hyb plate.

4. Transfer Reactions from Frag/Label Plate to Hyb Plate

- A. Transfer 8 μ L each reaction from the Frag/Label plate to the Hyb plate (total volume 100 μ L).
- B. Seal, vortex, spin.
- C. Plate to thermal cycler; run DMET Plus Denature.

5. Import Sample Information

- A. Scan array barcodes into sample information spreadsheet.
- B. Upload sample information into AGCC.

6. Load Sample onto Arrays – Work with 16 Arrays at a Time

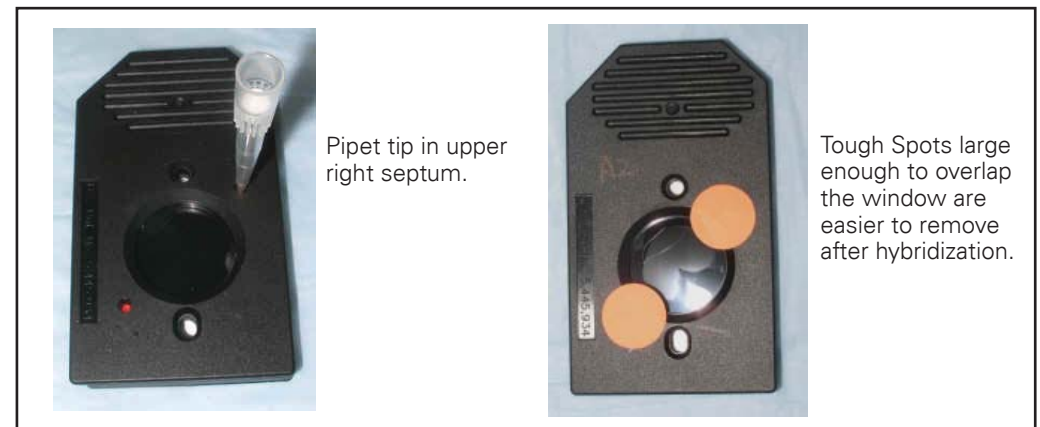
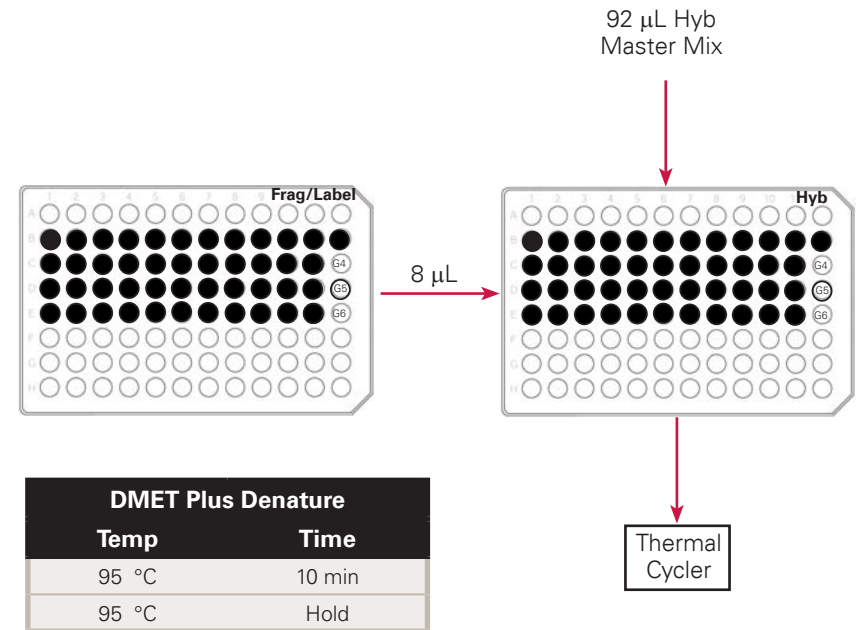
- A. Remove plate from thermal cycler, cool for 2 min, and spin.
- B. Return to aluminum block; hyb from the block.
- C. Inject 95 μ L each sample onto an array.
- D. Remove pipet tip and cover both septa with Tough Spots.
- E. When 16 arrays are loaded, place in hybridization oven.
- F. When all arrays in oven, allow to hybridize 16 to 18 hr.

7. To prepare for the next stage, transfer the Stain and Hold Buffers from -20 °C to 4 °C to thaw overnight.

Reagent Lot Numbers:

Hybridization Solution: _____

Oligo Control Reagent: _____



Quick Reference Card

DMET Plus Protocol

Stage 8 – Washing, Staining, and Scanning Arrays

In the Post-Amp Lab

1. Mix Stain and Hold Buffers

- A. Ensure buffers are thawed.
- B. Invert each tube 5X.

2. Prime the Fluidics Station

- A. Install Wash Solution A and B bottles.
- B. Fill dH₂O container.
- C. Empty waste container.
- D. In AGCC software, run the PRIME_450 script.

3. Prepare SAPE Stain Solution

- A. Add 90 μ L of SAPE to Stain Buffer tube.
- B. Mix by inverting tube 5X.
- C. Keep protected from light (do not place on ice).

4. Setup Software and Fluidics Station

- A. Select protocol DMET-Plus_169_v2.
- B. In position 1: amber tube with 300 μ L SAPE Stain Solution.
- C. In position 2: clear tube with 300 μ L Hold Buffer.
- D. In position 3: empty tube.
- E. Wash and stain arrays.
- F. When finished, run the Shutdown_450 protocol.

5. Scan Arrays

- A. Warm up scanner for 10 min.
- B. Remove arrays from fluidics station and inspect for bubbles.
- C. Cover both septa with Tough Spots (do not overlap window)
- D. Load arrays onto scanner and scan.

Reagent Lot Numbers:

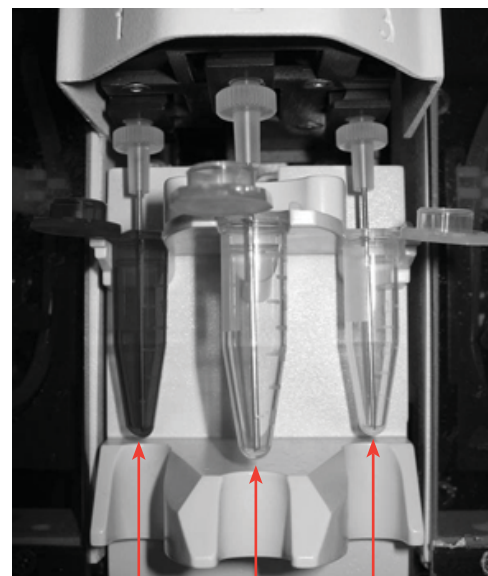
Wash Solution A: _____

Wash Solution B: _____

Stain Buffer: _____

Hold Buffer: _____

SAPE: _____



Position 1
300 μ L SAPE Stain
Solution
(amber tube)

Position 3
Empty tube

Position 2
300 μ L Hold Buffer



Do not overlap window with Tough Spots. Overlapping can cause the array to get stuck in the scanner.