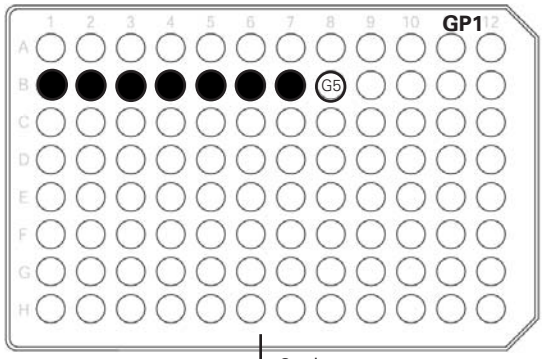
Quick Reference Card

DMET Plus Starter Pack 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)

(G5) = 17 µL gDNA control 5

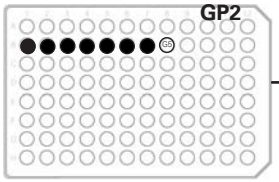
◯ = Empty well

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

2 µL

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

To each well:
A. Add 10 µL 1X TE Buffer.
B. Transfer 2 µL each sample/control from plate GP1.
C. Seal, vortex and spin down.



5 µL

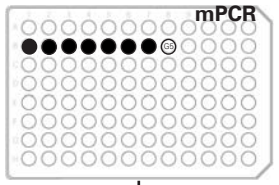
3. Prepare mPCR Master Mix (mix 5X with P1000 set to 600 µL; do not vortex)

mPCR MASTER MIX Reagent	Per Sample	8 Samples (> 20% extra)	Lot Number
QIAGEN Multiplex PCR Master Mix	25 µL	425 µL	
mPCR Primer Mix (3 µM)	5 µL	85 µL	
5X Q-Solution	5 µL	85 µL	
RNase-free Water	10 µL	170 µL	
Total Volume	**45 µL**	**765 µL**	—

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

4. Prepare Plate mPCR.

To each well:
A. Add 45 µL mPCR Master Mix.
B. Transfer 5 µL each sample/control from plate GP2.
C. Seal, vortex and spin down.



5. Plate mPCR onto thermal cycler.

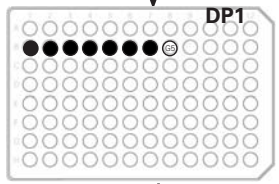
DMET Plus mPCR		
Temp	**Time**	**Cycles**
94 °C	15 min	—
95 °C	30 sec	35 Cycles
60 °C	90 sec	
72 °C	45 sec	
72 °C	3 min	—
4 °C	Hold	—

In the mPCR Staging Area

5 µL

6. Prepare Dilution Plate 1 (DP1).

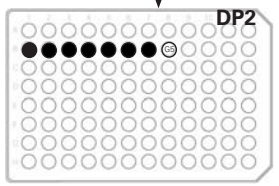
To each well:
A. Add 153 µL PCR Dilution Buffer.
B. Transfer 5 µL each sample/control from plate mPCR.
C. Mix slowly 10X with P100 set to 80 µL. Do not blow out pipet tips.



5 µL

7. Prepare Dilution Plate 2 (DP2).

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



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DMET Plus Starter Pack Protocol
Stage 2 – Anneal

In the Pre-Amp Lab

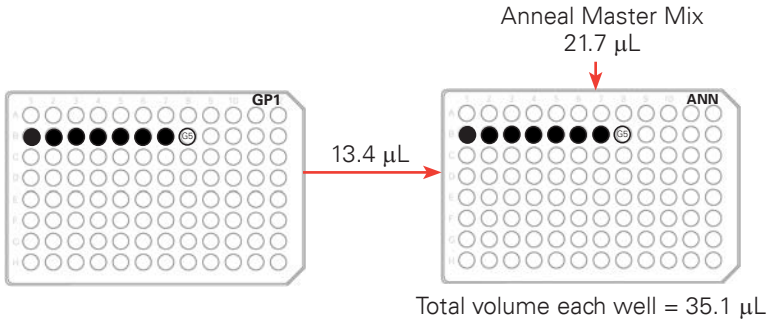
1. Prepare the Anneal Master Mix (mix 5X with P1000 set to 900 µL; do not vortex).

Anneal MASTER MIX Reagent	Per Sample	8 Samples (> 8 rxn 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:
A. Add 21.7 µL Anneal Master Mix to each sample and control.
B. Transfer 13.4 µL each sample from GP1.



Total volume each well = 35.1 µL

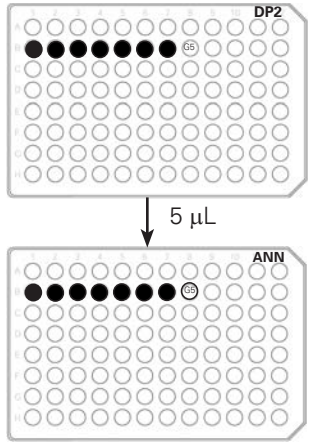
3. Seal and transfer plate ANN to the mPCR Staging Area.

In the mPCR Staging Area

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

To each gDNA sample and control:
Add 5 µL diluted mPCR product from plate DP2

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:
A. Remove plate and cool in aluminum block on ice for 2 min.
B. Aliquot to strip tubes, 10 µL each tube.
C. Add 5 µL DMET MIP Panel to each reaction.
D. Seal plate, vortex, spin down.
E. Put plate back on thermal cycler and resume the program.
F. 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

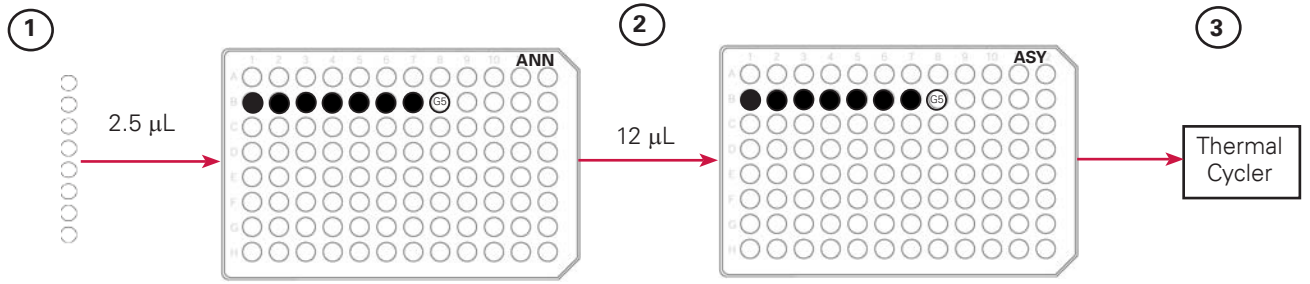
Total volume per well: 45.1 µL

Quick Reference Card

DMET Plus Starter Pack Protocol
Stage 3 – Gap Fill through Amplification

In the Pre-Amp Lab

1. **Add Gap Fill Mix to Anneal Plate.**
   A. Add 2.5 µL Gap Fill Mix to each well.
   B. 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 °C,** chill plate for 2 min, spin, add Exo Mix. Seal, vortex spin. Resume program.
6. **When thermal cycler reaches 60 °C,** chill plate for 2 min, spin, add Universal Amp Mix. Seal, vortex spin. Resume program.



Gap Fill Mix
1. Spin Gap Fill Mixes 1 and 2.
2. To an Eppendorf tube:
   A. Slowly add 190 µL Gap Fill Mix 2.
   B. Slowly add 10 µL Gap Fill Mix 1.
   C. 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
1. Vortex and spin.
2. Aliquot to strip tubes, 25 µL each tube.

Lot number:

_____

Exo Mix
1. Spin (no vortex).
2. Aliquot to strip tubes, 25 µL each tube.

Lot number:

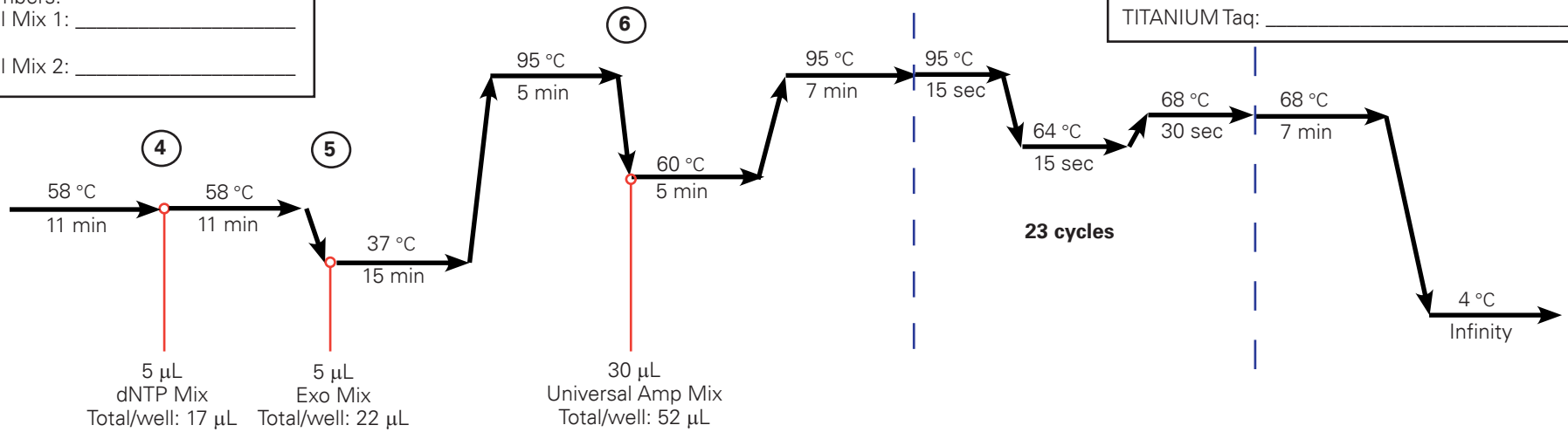
_____

Universal Amp Mix
1. Vortex and spin Universal Amp Mix tube.
2. Spin Cleavage Enzyme and TITANIUM Taq.
3. To a 1.5 mL tube, add:
   - 854.4 µL Universal Amp Mix
   - 10.7 µL Cleavage Enzyme
   - 29.9 µL Taq Polymerase
4. Mix 10X with P1000 set to 500 µL.
5. Pour into reagent reservoir.

Lot numbers:
Universal Amp Mix: _____

Cleavage Enzyme: _____

TITANIUM Taq: _____



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DMET Plus Starter Pack Protocol
Stage 4 – PCR Cleanup and First QC Gel

In the Post-Amp Lab

1. Add PCR Cleanup Mix

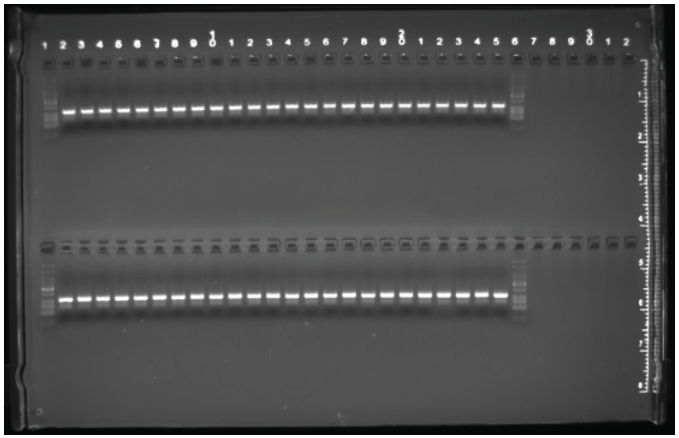
A. Spin down.
B. Aliquot to strip tubes, 15 µL each tube.
C. Add 2.5 µL to each reaction on ASY plate (total volume 54.5 µL).
D. Seal, vortex, and spin.
E. Plate to thermal cycler; run DMET Plus Cleanup.

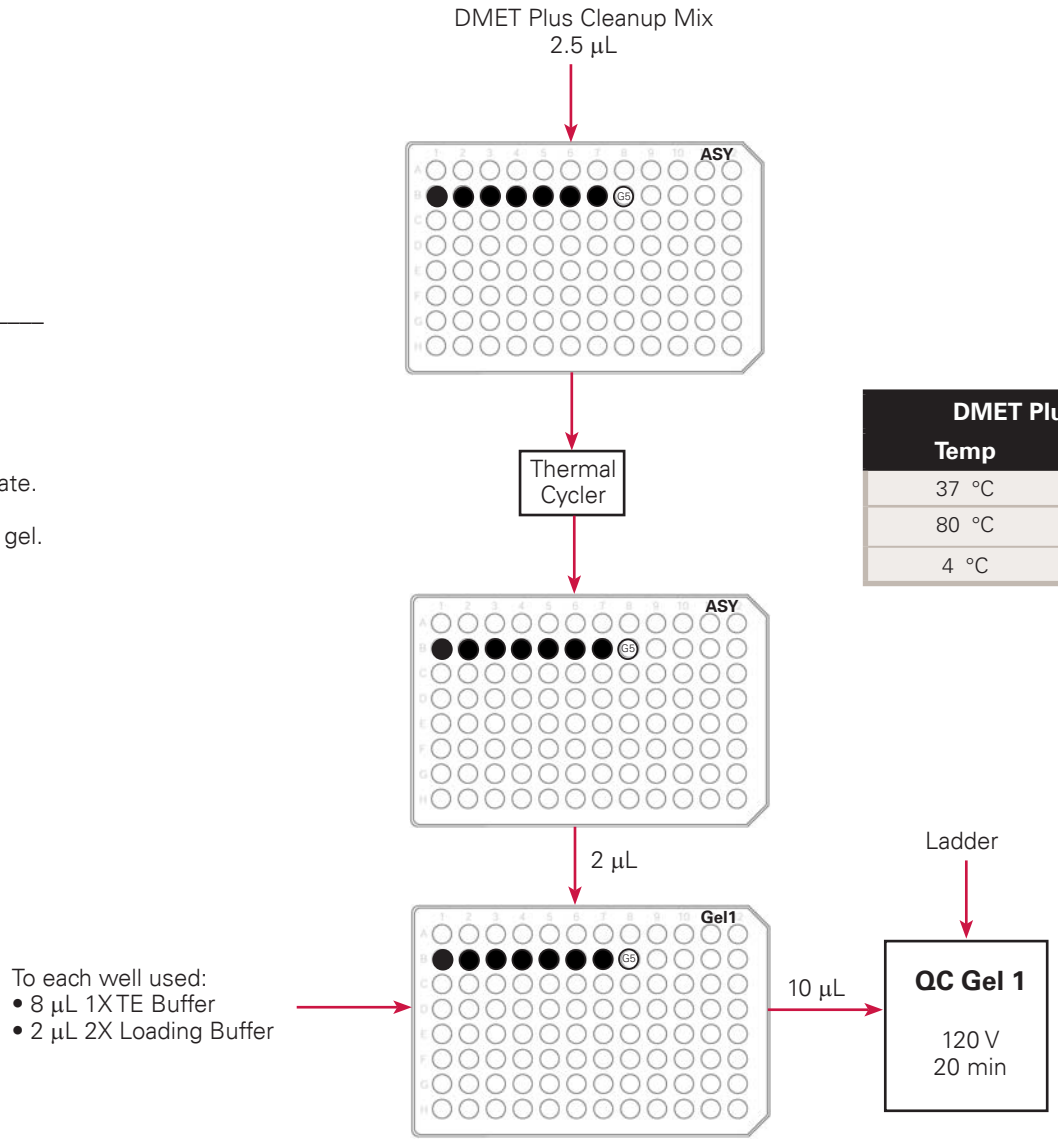
PCR Cleanup Mix Lot Number: _____

2. QC Gel 1

A. Remove plate from thermal cycler and spin.
B. Aliquot 8 µL 1XTE Buffer to the gel plate.
C. Add 2 µL of 2X Loading Buffer.
D. Transfer 2 µL each reaction from the ASY plate to the gel plate.
E. Seal, vortex, spin the gel plate.
F. Load 10 µL each reaction and the ladder onto a 3% agarose gel.
G. Run the gel at 120 V for 20 min.

Example of good QC Gel 1





DMET Plus Cleanup	
Temp	**Time**
37 °C	15 min
80 °C	15 min
4 °C	Hold

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DMET Plus Starter Pack Protocol
Stage 5 – Fragmentation and Second QC Gel

In the Post-Amp Lab

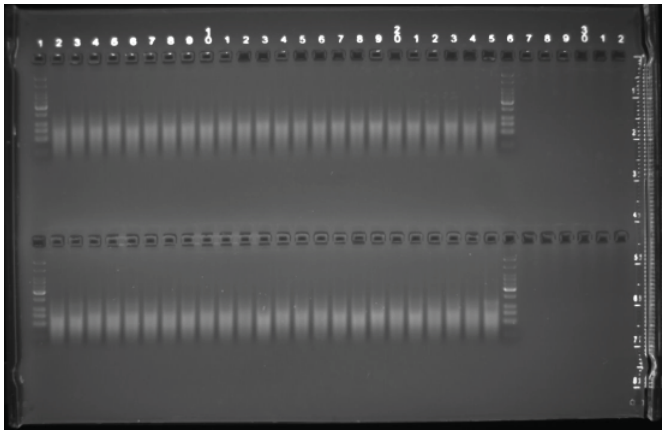
1. Transfer Reactions to Frag/Label Plate
A. Transfer 25 µL each reaction from the ASY to the Frag/Label plate.

2. Prepare the Fragmentation Master Mix
A. To a 1.5 mL Eppendorf tube add:
- 536 µL Post-Amp Water
- 60 µL Fragmentation Buffer

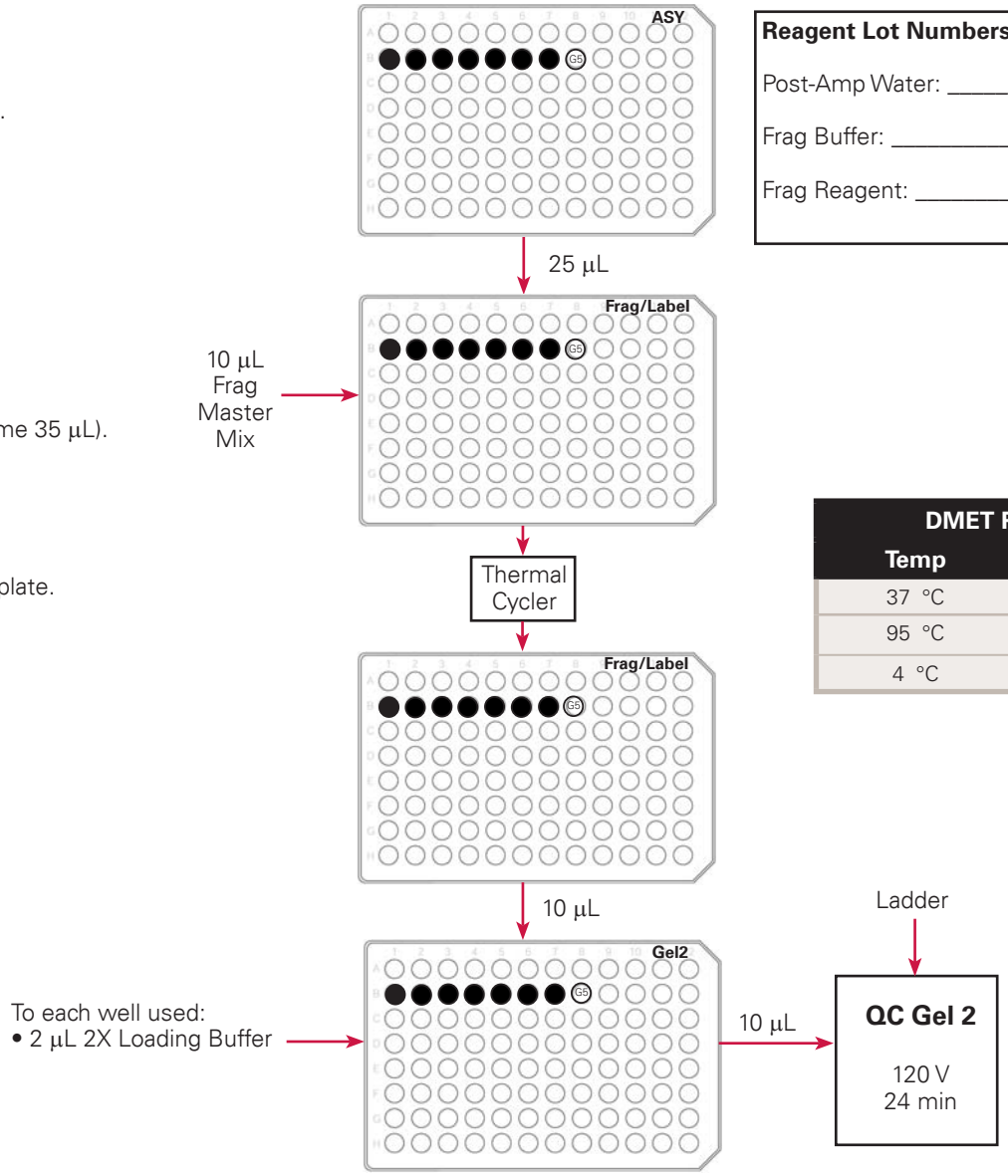
B. Cool on ice for 5 min.
C. Add 4.1 µL Fragmentation Reagent.
D. Vortex, spin and place on ice.
E. Aliquot to strip tubes, 45 µL each tube.

3. Add the Fragmentation Master Mix to the Frag/Label Plate
A. Add 10 µL Fragmentation Master Mix to each reaction (total volume 35 µL).
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 µL each reaction from the Frag/Label plate to the gel plate.
C. Add 2 µL 2X Loading Buffer.
D. Seal, vortex, spin the gel plate.
E. Load 10 µL 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

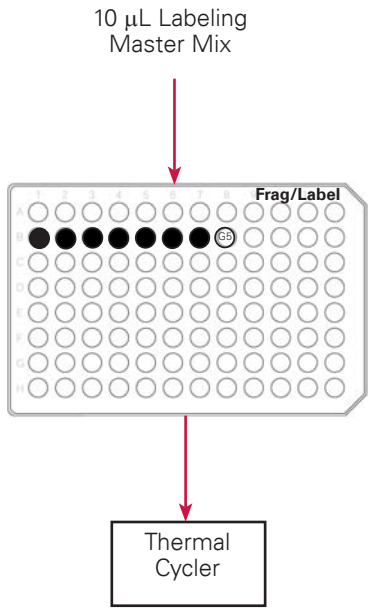
DMET Plus Starter Pack Protocol
Stage 6 – Labeling

In the Post-Amp Lab

1. Prepare the Labeling Master Mix
A. Prepare in 1.5 mL Eppendorf tube.
B. Vortex, spin and place on ice.
C. Aliquot to strip tubes, 45 µL each tube.

LABELING MASTER MIX			
Reagent	**Per Sample**	**8 Samples (> 8 rxn 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
A. Add 10 µL Labeling Master Mix to each reaction (total volume 35 µL).
B. Seal, vortex, spin.
C. Plate to thermal cycler; run DMET Plus Label.



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

Quick Reference Card

DMET Plus Starter Pack 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 µL pipet tip in upper right septum.

3. Prepare the Hyb Master Mix and Aliquot to Hyb Plate
A. To the Hybridization Solution tube, add 25 µL 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/dispensing 3X.
E. Aliquot 92 µL Hyb Master Mix to the Hyb plate.

4. Transfer Reactions from Frag/Label Plate to Hyb Plate
A. Transfer 8 µL each reaction from the Frag/Label plate to the Hyb plate (total volume 100 µL).
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.

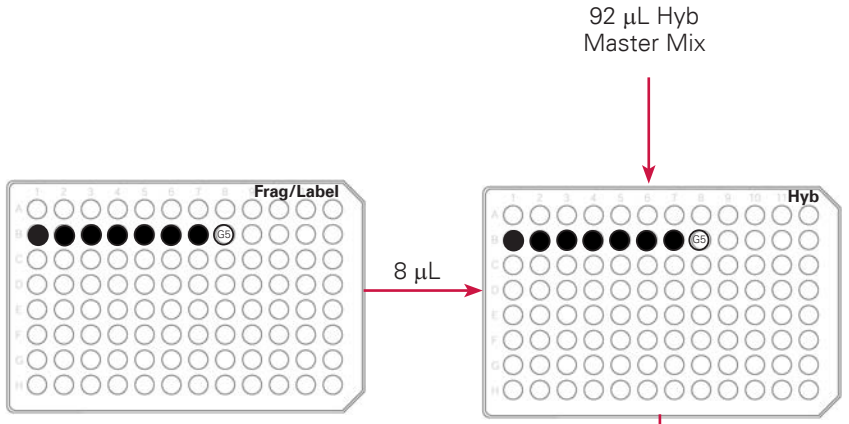
6. Load Sample onto Arrays – Work with 16 Arrays at a Time
A. Remove plate from thermal cycler, cool for 2 min, and spin.
B. Return to aluminum block; hyb from the block.
C. Inject 95 µL each sample onto an array.
D. Remove pipet tip and cover both septa with Tough Spots.
E. When 16 arrays are loaded, place in hybridizaton 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: _____



DMET Plus Denature	
Temp	**Time**
95 °C	10 min
95 °C	Hold

Pipet tip in upper right septum.

Tough Spots large enough to overlap the window are easier to remove after hybridization.

Quick Reference Card

DMET Plus Starter Pack Protocol
Stage 8 – Washing, Staining and Scanning Arrays

In the Post-Amp Lab

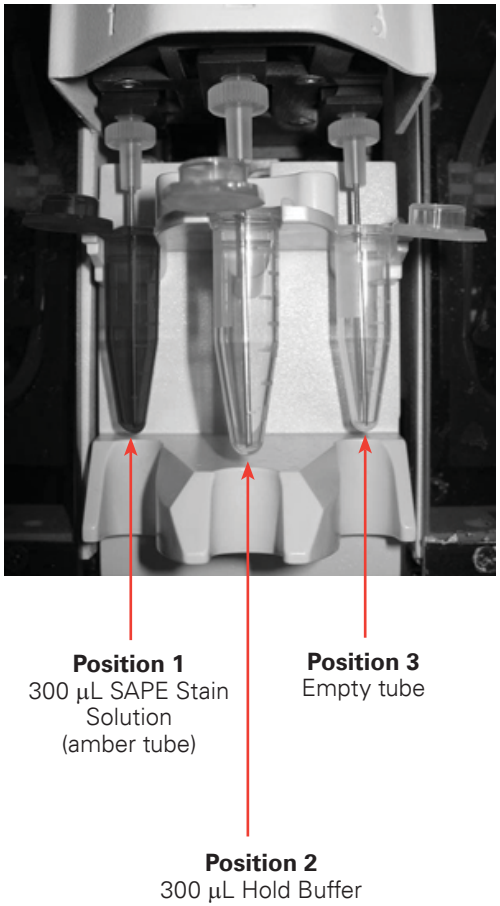
1. Mix Stain and Hold Buffers
A. Ensure buffers are thawed.
B. Invert each tube 5X.

2. Prime the Fluidics Station
A. Install Wash Solution A and B bottles.
B. Fill dH_2O container.
C. Empty waste container.
D. In AGCC software, run the PRIME_450 script.

3. Prepare SAPE Stain Solution
A. Add 22.5 µL 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 µL SAPE Stain Solution.
C. In position 2: clear tube with 300 µL 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.



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

Reagent Lot Numbers:

Wash Solution A: _____

Wash Solution B: _____

Stain Buffer: _____

Hold Buffer: _____

SAPE: _____