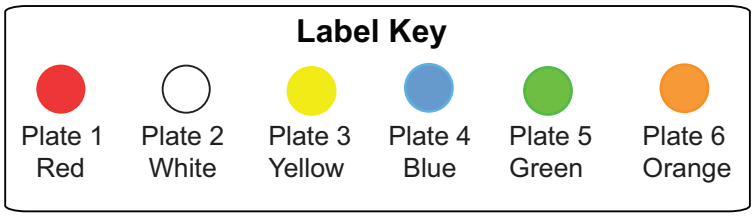
Fluidigm® Access Array Multiplex 20X Primer Solution Preparation Quick Reference

1 Preparing Stock Multiplex Primer Mix

1 Label each plate containing individual primer pairs with the provided plate stickers as follows:



2 Prepare and label a new 96-well plate for the Stock Multiplex primer mix.

3 In a DNA-free hood, prepare the Stock Multiplex primer mix for up to 480 individual primer pairs as shown in the Multiplexed Primer Stock Map on the other side of this document. The figure shows the source of the individual primer pairs and the destination location in the Stock Multiplex primer plate.

Using an 8-channel pipetter, combine **5 µl** from each of the 12 columns in the source plate to the designated single column in the destination plate. All 12 wells should be combined--even if they contain primer pairs or water--to maintain the appropriate primer concentrations. Primer pairs are provided mixed at **60 µM** each primer. After combining all 12 wells into the Multiplexed Primer Stock Plate, each primer is diluted to **5 µM**.

Repeat this procedure for all 48 Stock Multiplex primer mixes across the 6 primer plates of individual primer pairs.

NOTE Each well in the Multiplexed Primer Stock Plate will contain up to 12 Forward Primers and 12 Reverse Primers.

2 Preparing 20X Primer Solutions

1 In a DNA-free hood, prepare the 20X primer solutions as shown in the table below. The table shows the primer dilution for one well of the Stock Multiplex primer mix plate. This is repeated for all 48 wells. These will be loaded into the inlets of a 48.48 Access Array™ IFC.

Dilution of Stock Multiplex primers

Component	Volume (µL)	Final Concentration
Stock Multiplex Primer Mix (5 µM)	20	1 µM per Primer
20X Access Array Loading Reagent	5	1X
DNA Suspension Buffer	75	

2 Seal the 20X multiplex dilution plate with an adhesive seal and vortex for a minimum of 20 seconds, and centrifuge for 30 seconds to spin down all components.

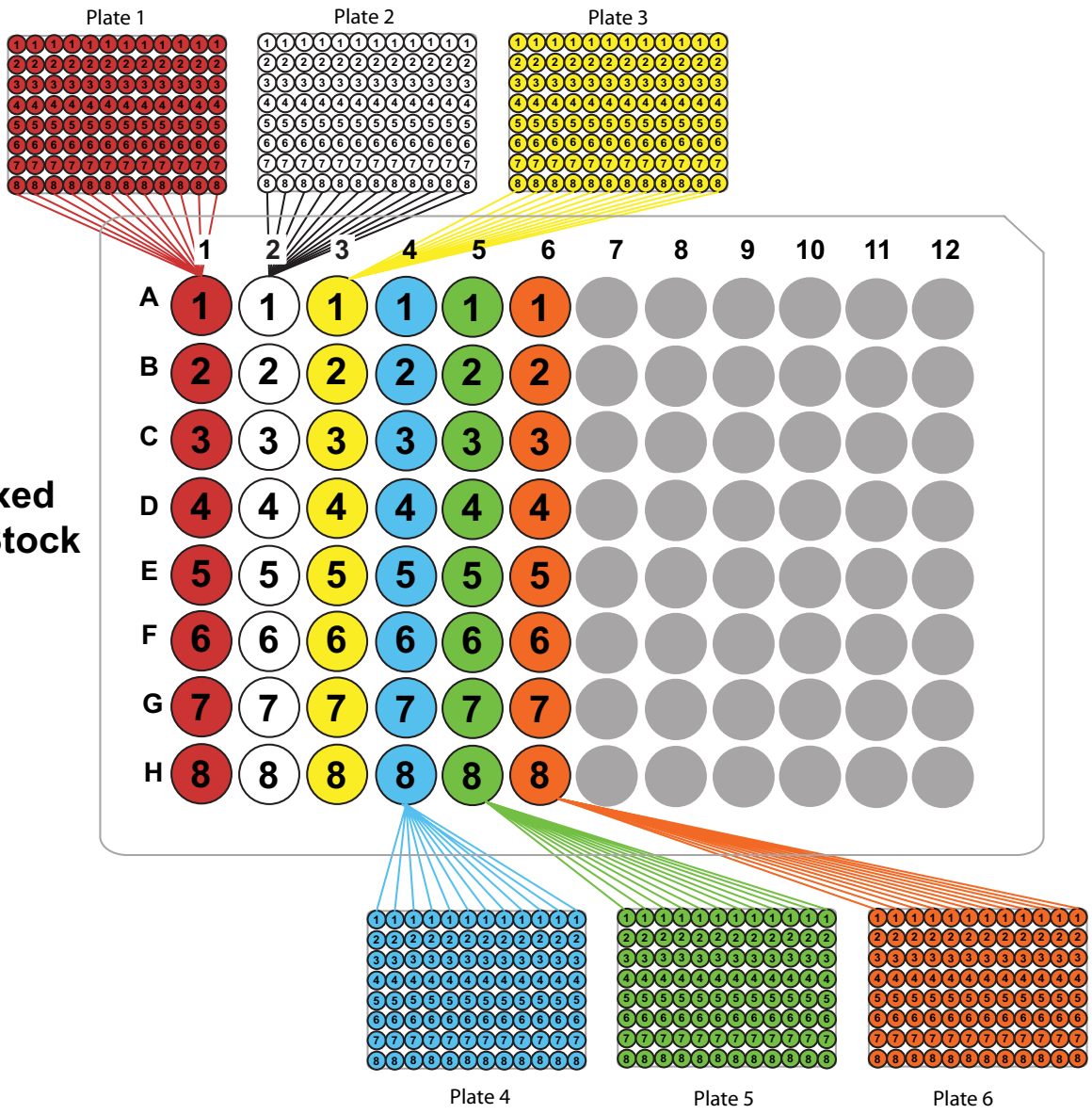
3 Store all primer plates at -15ºC to -85ºC.

If you are performing sequencing on the **454 GS FLX** or **GS Junior Sequencers**, see Advanced Development Protocol 21, *Multiplex Amplicon Tagging for 454 Titanium Sequencing on the 48.48 Access Array IFC* (PN 100-2005) for next steps.

If you are performing sequencing on the **Illumina GAII**, **HiSeq**, or **MiSeq sequencers**, see the chapter on Multiplex Amplicon Tagging for the Illumina on the 48.48 Access Array IFC in the *Fluidigm® Access Array™ System User Guide for Illumina Sequencing Platform* (PN 100-3770) for next steps.

Use the actual size Multiplexed Primer Stock Map on the other side of this document as a guide when loading the plate.

Multiplexed Primer Stock Map



Technical Support

TELEPHONE

Within the United States: 1-866-358-4354

Outside the United States: 1-650-266-6100

EMAIL

techsupport@fluidigm.com

Fluidigm®