User Bulletin

Affymetrix Targeted Genotyping System

UB No. 1

October 2007

Topic: Quick Reference Card Modifications and HiPlex Protocol

This user bulletin summarizes the changes incorporated into the new set of quick reference cards (QRCs) that describe how to perform the MIP Assay Protocol. These instructions now include performing the assay with panels equal to or greater than 25K-plex.

The instructions in this new set of QRCs (P/N 690053, rev 2) supersedes the information in the following documents:

- The previous set of QRCs available as P/N 690053, rev 1.
- The *GeneChip® Scanner 3000 Targeted Genotyping System User Guide*, P/N 702126, rev 1.

The new set of QRCs is available for download from www.affymetrix.com.

IMPORTANT: The changes described in this User Bulletin are summaries only. Complete details for each change are not included. Print a new set of cards and carefully review each change. Be sure to replace the rev 1 QRCs with updated cards in your labs.

Summary of Changes

The title, part number and revision number of each QRC along with a summary of the changes made to the card is listed below.

Chilling Aluminum Blocks

Always chill aluminum blocks in a 4°C refrigerator. Do not chill in a –20°C freezer. Chilling to –20°C may cause reagents to refreeze.

Create a Project (P/N 702167, rev 2)

Assay Panel and Genotype Settings Files

These files are no longer provided on CD-ROM with your first assay panel shipment. These files must now be downloaded from www.affymetrix.com.

Normalizing Samples

HiPlex protocol gDNA normalization requirements added.

Stage 1 – Design an Anneal Plate (P/N 702168, rev 2)

No changes made to the instructions on this card.

Stage 2 – Anneal, Card 1 of 2 (P/N 702169, rev 2)

Special Edition Panels

All references to Special Edition Panels have been removed.

Volumes

10K/20K volumes for reagents and pipetting changed to ≥ 10K.

Thawing Enzyme A

- Chill aluminum blocks in a 4°C refrigerator. Do not chill in a –20°C freezer.
- Enzyme A must be thawed before adding to the Anneal Cocktail. Instructions for thawing Enzyme A have been added to the QRC.

Preparing the Anneal Cocktail

Modifications made to reflect thawing Enzyme A.

Aliquoting the Anneal Cocktail

If using electronic pipettes, use a 24-channel P100 electronic pipette to aliquot the Anneal Cocktail for 3K/5K assay panels.

Stage 2 – Anneal, Card 2 of 2 (P/N 702169, rev 2)

Special Edition Panels

All references to Special Edition Panels have been removed.

Volumes

10K/20K volumes for reagents and pipetting changed to ≥ 10K.

Stage 3 – Plan and Run Assay Plates (P/N 702178, rev 2)

No changes made to the instructions on this card.

Stage 4 – Gap Fill, dNTP, Ligate, Invert, First PCR, Card 1 of 3 (P/N 702170, rev 2)

Preparing the Gap Fill Mix

Added instruction to place three chilled aluminum blocks on ice (chilled in 4°C refrigerator).

Adding Gap Fill Mix

Place Anneal Plate in chilled aluminum block on ice and cool for 2 minutes. Then spin down and return to the aluminum block before adding the Gap Fill Mix.

Thermal Cycler Program

The thermal cycler program for assay panels ≥ 25K was added.

Stage 4 – Gap Fill, dNTP, Ligate, Invert, First PCR, Card 2 of 3 (P/N 702170, rev 2)

No changes made to the instructions on this card.

Stage 4 – Gap Fill, dNTP, Ligate, Invert, First PCR, Card 3 of 3 (P/N 702170, rev 2)

The thermal cycler program for assay panels ≥ 25K was added.

Stage 5 – Second PCR, Card 1 of 2 (P/N 702171, rev 2)

Thawing the Reagents

Thaw reagents on bench top at room temperature until defrosted; then place on ice.

Transferring Reactions to Label Plates

To aliquot Allele Tube mixes to the Label Plate, work two rows at a time using a 24-channel P100 pipette.

Changed *For 10K/20K assay panels* to *For ≥ 10K assay panels*.

First Quality Control Gel

Mix 7 µL of each sample with 4 µL of loading buffer.

Load 2 µL of 100 bp marker in a single lane.

Stage 5 – Second PCR, Card 2 of 2 (P/N 702171, rev 2)

Use a 24-channel P100 pipette to aliquot Allele Tube mixes to the Label Plate.

Under Thermal Cycler Programs: Changed *10K/20K assay panels* to *≥ 10K assay panels*.

Stage 6 – Target Digest, Card 1 of 2 (P/N 702172, rev 2)

No changes made to the instructions on this card.

Stage 6 – Target Digest, Card 2 of 2 (P/N 702172, rev 2)

No changes made to the instructions on this card.

Stage 7 – Sample Hybridization (P/N 702173, rev 2)

Thawing the Hyb Cocktail

Removed instructions to wrap tube of Hyb Cocktail in aluminum foil while thawing.

Added 50K Plus Hyb Mix.

Add Hyb Cocktail

Added 50K Plus Hyb Mix.

Modify the Meg Denature Thermal Cycler Program

Modify the Meg Denature program to run 6 min at 95°C, then Hold at 95°C.

Denaturing the Samples

After the 6 minute denature, place the Hyb Plate in a chilled aluminum block on ice, cover with aluminum foil, and cool for 2 minutes.

Spin down the plate, return it to the aluminum block, and leave on the block while loading samples onto arrays.

Stage 8 – Stain and Wash Arrays (P/N 702174, rev 2)

No changes made to the instructions on this card.

Stage 9 – Scan Arrays (P/N 702175, rev 2)

No changes made to the instructions on this card.

Manually Gridding Arrays (P/N 702177, rev 2)

Instructions added for manually gridding arrays with poorly defined corners.

MIP Assay Protocol Barcodes (P/N 702176, rev 2)

No changes made to the instructions on this card.

P/N 702578, Rev 1