

Supplemental Table 2. Variations Between Laboratories in Tissue Handling and RNA Preparation for Microarray Analysis.

Step	Process	Site 1 UPitt	Site 2 VCU	Site 3 Cogenics	Site 4 Stanford
1	Tumor isolation	<ul style="list-style-type: none"> ▪ RNAlater®-ICE ▪ Microscope, scalpel 	N/A	N/A	N/A
2	Tissue homogenization	Omni General Laboratory Homogenizer (GLH)	Cryostat, (10 µm slices) into TRIzol	Omni GLH	Fisher Scientific PowerGen 125
	Total RNA extraction	<ul style="list-style-type: none"> ▪ Ambion <i>mirVana</i>™ miRNA Isolation Kit ▪ QIAGEN RNeasy Midi/Mini Kits 	<ul style="list-style-type: none"> ▪ TRIzol ▪ QIAGEN RNeasy Mini Kit 	QIAGEN RNeasy Mini Kit	QIAGEN RNeasy Midi/Mini Kits
	Total RNA quantity used	1 µg	5 µg	1 µg	1 µg
	Total RNA quality for test (A ₂₆₀ /A ₂₈₀ ; 18S/28S Yes/No)	>1.8 Yes	>1.8 Yes	>1.8 Yes	>1.8 Yes, Agarose Gel Electrophoresis only
3	cDNA synthesis	GeneChip® One-Cycle cDNA Synthesis Kit (Manufactured by Invitrogen for Affymetrix)			
	cDNA cleanup	Affymetrix Sample Cleanup Module (Manufactured by QIAGEN for Affymetrix)			
	In vitro transcription	GeneChip® Expression 3'-Amplification Reagents for IVT Labeling (IVT Labeling Kit with MEGAscript® reagents; Manufactured by Ambion for Affymetrix)			
	cRNA cleanup	Affymetrix Sample Cleanup Module			
4	Fragmentation	5X Fragmentation Buffer (Affymetrix)			
5	Hybridization	GeneChip® Hybridization, Wash, and Stain kit	Laboratory-prepared reagents	GeneChip® Hybridization, Wash, and Stain kit	GeneChip® Hybridization, Wash, and Stain kit
	GeneChip® array	<ul style="list-style-type: none"> ▪ Human Genome U133A (HG-U133A) (Affymetrix) ▪ Pathchip™ (Manufactured by Affymetrix for Pathwork Diagnostics) 	<ul style="list-style-type: none"> ▪ HG-U133A ▪ Pathchip 	<ul style="list-style-type: none"> ▪ HG-U133A ▪ Pathchip 	Pathchip
	Fluidics station	Affymetrix GeneChip® Fluidics Station 450 (FS450)	Affymetrix FS450	Affymetrix FS450	Affymetrix FS450 Dx

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5 cont.	Fluidics script	<ul style="list-style-type: none"> ▪ FS450_0001 ▪ FS450_0004 	<ul style="list-style-type: none"> ▪ FS450_EukGE-WS2v4 ▪ FS450_EukGE-WS2v5 	<ul style="list-style-type: none"> ▪ FS450_0001 ▪ FS450_0004 	FS450_0001
	Hybridization oven/ temperature/ rotation speed	Affymetrix Hybridization Oven 640 (HO640) 45 °C 60 RPM	Affymetrix HO640 45 °C 60 RPM	Affymetrix HO640 45 °C 60 RPM	Sheldon Mfg. Inc. SHEL LAB Hybridization Oven model #1012AG 45 °C 20 RPM
	Wash and staining	GeneChip® Hybridization, Wash, and Stain kit	Laboratory-prepared reagents	GeneChip® Hybridization, Wash, and Stain kit	GeneChip® Hybridization, Wash, and Stain kit
	Microarray Scanner	GeneChip® Scanner (GCS) 3000 6G* with Autoloader	GCS 3000 6G* with Autoloader	GCS 3000 6G* with and without Autoloader	GCS 3000 Dx
6	GeneChip® Operating Software	Version 1.4	Version 1.4	Version 1.4	Version 1.1.3
	Pathchip™ Tissue of Origin Software	Version 21.2			

RPM, revolutions per minute.