SurePrint G3 Human Gene Expression Array (Agilent)

Pediatric Preclinical Testing Program (PPTP) – Gene Expression

*Protocols were performed at XXX

The RNA extraction was performed according to Qiagen manufacturer's protocol (RNeasy kit).

The nucleic acid labeling was performed according to the manufacturer's protocol for One-Color Microarray-Based Gene Expression Analysis (Agilent Technologies). The Low Input Quick Amp Labeling Kit, One-Color generated fluorescent cRNA with a sample input RNA range between 10ng and 200ng of total RNA or a minimum of 5ng of poly A+ RNA for one-color processing. The method uses T& RNA Polymerase Blend (red cap)6 which simultaneously amplifies target material and incorporates Cyanine 3-CTP.

The nucleic acid hybridization to array was performed according to the manufacturer's protocol for One-Color Microarray-Based Gene Expression Analysis (Agilent Technologies). Briefly, the 10x blocking agent was prepared by adding 500ul of nuclease-free water to the 10x agent supplied with the kit, mixed on a vortex and centrifuged for 5-10 seconds.The RNA fragmentation reaction was performed at 60°C for 30 minutes, after which the samples were colled on ice for one minute and 2x Hi-RPM Hybridization Buffer was added to stop the reaction. These samples were further mixed, spun for 1 minute at room temperature at 13,000xg, placed on ice and loaded on array. The arrays were hybridized at 65°C for 17 hours. This step was followed by microarray slides wash with Gene Expression Wash Buffers I and II.

The array scanning was performed according to the manufacturer's protocol for One-Color Microarray-Based Gene Expression Analysis (Agilent Technologies). The assembled slide holders were put into the scanner cassette, after which the appropriate scanner protocol is selected and ran. In order to extract information from probe features from microarray scan data, the Feature Extraction process is performed using the software provided at Agilent web-site.