Supplemental Methods 1. RNA Extraction from FFPE Tissue

RNeasy FFPE kit, catalog number 73504 from Qiagen Valencia, CA 91355

Ten µM FFPE tissue sections in micro-centrifuge tubes were deparaffinized by mixing with xylene and washing with ethanol after residual xylene was removed. After all the residual ethanol was evaporated, proteinase K buffer and proteinase K were added to the samples and incubated at 56ºC for 15 min and then 80 ºC for 15 min. After incubation, the lower, uncolored phase was transferred into a new micro-centrifuge tube and incubated on ice for 3 minutes. The supernatant was transferred to a new micro-centrifuge tube without disturbing the pellet. DNase Booster buffer and 10µl of DNase I stock was added to the sample. The mix was incubated at room temperature for 15 minutes to remove DNA. RBC buffer was added to the mix to adjust binding conditions, followed by 100% ethanol to precipitate RNA. RNA was added to RNeasy MinElute spin columns, washed and eluted in 14-30µl of RNase-free water.

Supplemental Methods 2. Real-Time PCR Protocol

High Capacity RNA-to-cDNA™ Kit, 4387406

TaqMan PreAmp Master Mix Kit 4384267

High Capacity RNA-to-cDNA Kit from Applied Biosystems/Life technology was used to generate cDNA for real-time (RT)-PCR. Total RNA (300 ng) was mixed with 10µl of 2X reverse transcriptase buffer, 1µl enzyme mix and adjusted to a final volume of 20µl. The reaction was mixed and incubated at 37ºC for 60 minutes and then the reaction was stopped by heating to 95ºC for 5 minutes.

cDNA was stored at -20 ºC until analyzed. Six sets of 20X Taqman assays, including one house-keeping gene and five target genes were pooled together by adding 2 µl of each assay and then diluted with 1X TE buffer to a final volume of 200 µl. To perform the pre-amplification (preamp) reaction, 25 µl of preamp master mix was mixed with 12.5 µl of pooled assays and 12.5 µl of cDNA generated from the previous step. The mix was incubated at 95 ºC for 10 minutes for the initial step, and then at 95 ºC for 15 seconds and 60 ºC for 4 minutes for a total of 10 cycles. The preamp product was diluted 1:5 with TE buffer before use in the RT-PCR reaction. 5 µl of the diluted preamp product was mixed with 10 µl of 2X master mix and 1 µl of each 20X gene expression assay and this was adjusted to a final volume of 20 µl. The reaction mix was incubated in the RT-PCR cycler at 50 ºC for 2 minutes, then 95 ºC for 10 minutes, and then the start cycle at 95 ºC for 15 seconds, 60 ºC for 1 minute for a total of 40 cycles. Data were collected and analyzed.