

## **Dissections and Preparation Tissues**

Tissues for histologic analysis were isolated from euthanized mice after immediate ventricular perfusion with 50 ml of sterile Phosphate Buffer Saline (PBS) and 50 ml of 4% paraformaldehyde using a dissecting microscope and incubated overnight in 4% paraformaldehyde for 12 h and then washed briefly in 70% ethanol. Tissues were dehydrated using the Tissue-Tek Vacuum Infiltration Processor (Sakura Finetek USA Inc). Tissues were sequentially incubated at 35°C for 5 min in 70% EtOH, 10 min in 80% EtOH, 15 min in 95% EtOH, and then 5 min, 10 min and 15 min in 100% EtOH, and finally 30 min in Toluene. Tissues were then incubated in Paraffin once for 5 min and then sequentially 3 times for 10 min all at 58°C. Processed tissues were embedded in paraffin. 5- $\mu$ m sections, cut and mounted on glass slides, were hydrated through xylene and graded alcohol and equilibrated in PBS. Hematoxylin and eosin staining was performed as previously described (McMenamin, et. al.).

McMenamin, M.E., P. Soung, S. Perera, I. Kaplan, M. Loda, and W.R. Sellers. 1999. Loss of PTEN expression in paraffin-embedded primary prostate cancer correlates with high Gleason score and advanced stage. *Cancer Res* **59**: 4291-6.