As a procedural best practice, it is recommended that PCR products are cleaned up following initial amplification and prior to Sanger Sequencing. The goal of PCR product cleanup is to eliminate leftover primers and nucleotides that interfere with the sequencing reaction and lead to poor results. A speedy and efficient way to do this is with enzymes that digest excess PCR primers and degrade unincorporated nucleotides. The most effective and well referenced product for this is ExoSAP-IT reagent from Affymetrix®, which combines Exonuclease I primer digestion and Shrimp Alkaline Phosphatase nucleotide degradation into a single cleanup step. HT ExoSAP-IT Fast High-Throughput PCR Product Cleanup is the ideal solution in high volume labs with quick sample turnaround requirements. HT ExoSAP-IT Fast reagent has a 14 minute total protocol time, which is over 50% faster than standard ExoSAP-IT reagent. It is added directly to the PCR product for a 7 minute cleanup at 37°C, followed by a 7 minute inactivation at 80°C.

**Method**

The stability of HT ExoSAP-IT Fast reagent was assessed at the enzyme component level as well as in functional sequencing assays. Stability testing was conducted after 10 freeze thaw cycles and storage at -20°C, 4°C, and ambient temperatures. Additionally, HT ExoSAP-IT Fast reagent stability was measured in different container configurations including high volume large bulk vials, mid-volume 8-tube strips, and low volume 96-well plates.

**Results**

HT ExoSAP-IT Fast reagent displayed excellent stability in all formats tested including large bulk vials, 8-tube strips, and 96-well plates. The product showed no loss in function after 10 freeze thaw cycles, and remained stable at variable temperatures for extended periods of time compared to enzyme mix equivalents. The outstanding stability of HT ExoSAP-IT Fast reagent along with a >50% faster protocol make it ideal for high-throughput assays where robotic platforms are utilized and large volumes of samples are processed in successive testing runs.

PCR products cleaned up with HT ExoSAP-IT Fast reagent prior to automated sequencing displayed superior sequencing results compared to the un-cleaned equivalent (Figure 1). Collectively, these results clearly demonstrate the effectiveness of HT ExoSAP-IT Fast reagent to ensure highest quality sequences while keeping sample processing moving quickly and efficiently.

**Fig. 1. High quality sequencing data after treatment with HT ExoSAP-IT Fast reagent**

Sequencing of a 1KB PCR product cleaned with HT ExoSAP-IT Fast reagent (top) or un-cleaned (bottom). HT ExoSAP-IT Fast treatment prior to sequencing eliminates miscalls and improves sequencing quality scores (numbers and bars above sequence; >50, probability of error ≤0.001). Sequence shown is within the first 150 bases.