Introduction
Tumor heterogeneity and its impact on personalized medicine in the future
While ongoing efforts in cancer research are beginning to show a pathway to personalized medicine in the future, they are also uncovering the astonishing levels of genomic heterogeneity of cancer cells that make up a tumor [1]. A given tumor sample can have remarkably high levels of genetic complexity, and the identification of more rare mutations or subpopulations may, in the future, be key to guiding successful therapy [2]. However, traditional sequencing techniques present difficulties in detecting these rare variants. In addition, translational laboratories usually store tissue in formalin-fixed, paraffin-embedded (FFPE) material, which degrades DNA and provides it in only very small amounts, thus making genomic analysis of tumor DNA even more cumbersome.

Customer profile
Biomarker discovery at Heidelberg Medical Hospital
Dr. Volker Endris is a research associate in the Department of Pathology at Heidelberg Medical Hospital, heading the next-generation sequencing (NGS) facility. Dr. Endris and his colleagues work on translational molecular biology of different disease types, which includes research on known tumor markers resistant to radiation and chemotherapy, searching for predictive biomarkers in malignant solid tumor samples, and applying NGS technology to biomarker discovery. His current work focuses on the implementation of amplicon-based NGS for the molecular analysis of FFPE tumor material.

The challenge
Accelerating cancer marker discovery from FFPE samples
As cancer therapeutics rapidly shift toward genetics-driven stratification, there is an increasing demand for rapid, detailed molecular analysis of samples. Dr. Endris’s lab currently uses Sanger sequencing for each assay, due to the well-established reliability and accuracy of this approach. However, with the rise
“We found very high concordance between the Sanger and NGS data.”

Dr. Volker Endris

in demand for his translational studies, Dr. Endris felt the need to switch to multiplexed assays in his research to get a more comprehensive view of important variants while providing results in a timely, cost-effective fashion. However, in clinical research settings, tumor samples are often of low quality, with either low tumor-cell content or degraded DNA. This could pose a challenge for mutation discovery by next-generation sequencing.

The solution
Evaluating the Ion PGM™ System with FFPE samples
Dr. Endris and his colleagues tested the capacity of the Ion PGM™ System and the commercially available Ion AmpliSeq™ Cancer Hotspot Panel v2 to accurately sequence FFPE samples, and compared the results from Ion Torrent™ technology and traditional Sanger sequencing [3]. The team also evaluated the performance of custom panels developed with Ion AmpliSeq™ Designer. Dr. Endris found that Sanger sequencing could be conducted faster and was less costly for single-gene analysis, but Ion Torrent™ sequencing became more cost-effective and faster when analyzing a large number of exons/genes. NGS can thus enable more laboratories to perform research on the heterogeneity found in tumor samples.

Results
Detecting rare variants with the Ion PGM™ System
Dr. Endris found that the Ion PGM™ System showed very high concordance, even with FFPE tissue samples, with traditional Sanger sequencing. PCR-generated artifacts appeared to be low, indicating that technical challenges with FFPE material can be easily overcome with the Ion AmpliSeq™ technology and the Ion PGM™ System. The sensitivity analysis showed that with 2,000x coverage for the cancer research panels, NGS can detect mutations down to a 5% allele frequency. Evaluating mutations with low allele frequencies (below 5%) was technically difficult because of fixation artifacts in the source material, indicating the need to control the quality of sample material even when conducting NGS (Figure 1). Dr. Endris and his team may, in the future, use digital PCR to verify the low-frequency mutations detected by Sanger sequencing or NGS.

Cost-effective, rapid sequencing of more samples and amplicons
Methodologies for Sanger sequencing and NGS can be challenging to compare, especially when taking extra steps to work with difficult material like FFPE, Dr. Endris reported [3]. Costs of NGS depend on the total size of the library, chip capacity, and overall coverage, while Sanger sequencing...
costs are calculated on a per-sample basis. Three different chip sizes can be run on the Ion PGM™ System (Ion 314™, Ion 316™, and Ion 318™), enabling different multiplexing strategies to reduce per-sample costs. For example, following an 8-plex approach on a larger-capacity Ion 318™ chip vs. a singleplex approach on an Ion 314™ chip can reduce the per-sample cost by almost 40%. Because sequence output for NGS was 190 times greater than for Sanger sequencing, cost per amplicon was significantly lower using the Ion PGM™ System. Turnaround time per sample favored Sanger sequencing for a small number of amplicons, but as the number of amplicons analyzed increased the advantage quickly shifted to the Ion PGM™ System.

**Evaluating platform specificity**

Dr. Endris and his colleagues also tested the Ion PGM™ System against other NGS platforms, namely the Illumina MiSeq® system and Roche GS Junior™ system for research, using EGFR- and KRAS-positive samples, and compared results with other research centers. They found a very high concordance in calls between the three platforms and high reproducibility between the research centers, suggesting that panel sequencing on the Ion PGM™ System holds a lot of promise for reliable cancer marker discovery from FFPE samples.

**Opening the door to more connections**

NGS’s high output allows for a rapid comparison of many more alleles, making it possible to more accurately characterize disease states that were not possible using traditional methods. Dr. Endris and his colleagues recently published a paper on the genetic characterization of blastic plasmacytoid cell neoplasm (BPDCN), an orphan disease with a very aggressive clinical course that lacks standardized therapeutic options. The group studied about 50 common cancer genes in 33 BPDCN-positive FFPE samples using the Ion AmpliSeq™ Cancer Hostpot Panel v2 and the Ion PGM™ system. Dr. Endris hopes that the results of the study will further elucidate the pathobiology of BPDCN and enable development of more effective therapeutic options in the future [4].

**Conclusion**

The Ion Torrent™ NGS workflow: an ideal tool for more comprehensive cancer marker discovery

Working with degraded and difficult archived samples such as FFPE DNA can be a big challenge; however, the Ion AmpliSeq™ research panels require only 10 ng of input DNA, making them ideal for FFPE samples. While the per-sample sequencing costs and turnaround time per sample using Sanger sequencing are still superior to next-generation techniques, the higher number of samples and data produced by sequencing on the Ion PGM™ System point to the promise of NGS to analyze more samples at a much lower cost. This feasibility, while still for research use only, is valuable for applications such as cancer research, in which sequencing must capture a wide heterogeneity of markers and mutations in tumor cell samples. In addition, NGS has the capacity to enable biomarker discovery and mutation identification from low-volume and low copy number DNA, which has proved difficult with traditional Sanger sequencing. Studies using next-generation sequencing techniques are enabling a more comprehensive view of cancer gene mutations in a more timely fashion, thus bringing us to closer to making personalized cancer therapy a reality in the future.
References


Get more details about the Ion PGM™ System at [lifetechnologies.com/pgm](http://lifetechnologies.com/pgm)

Design your own panels at [ampliseq.com](http://ampliseq.com)