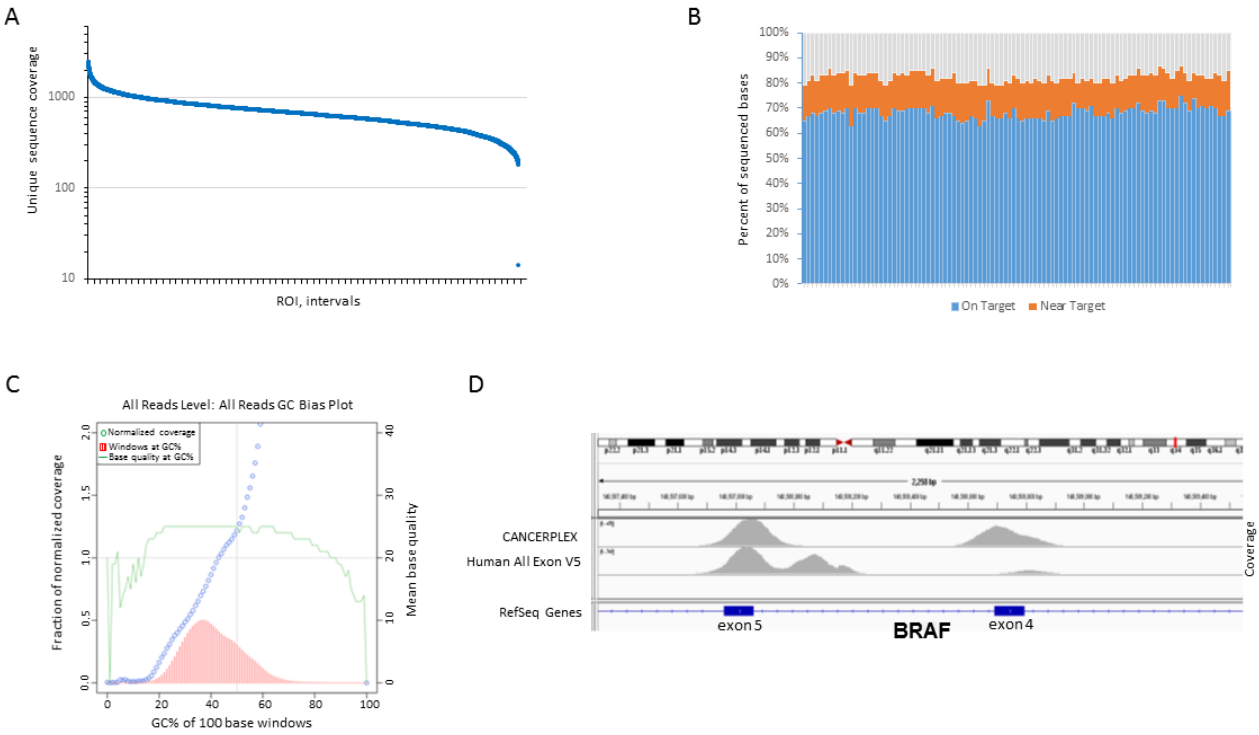
Supplemental Figure Legends

Supplemental Figure 1. Validation of sequence coverage across regions of interest. **(A)** High level sequence coverage is achieved across all regions of interest (ROI). **(B)** The majority of sequenced bases (~80) are aligned to or adjacent to ROI. **(C)** The GC bias plot demonstrates that the sequenced libraries produce uniform coverage regardless of the regional GC content. %GC content of 100bp windows (x-axis). Blue circles (normalized coverage), red lines (%GC of the ROI), green line (base quality across GC percentages).

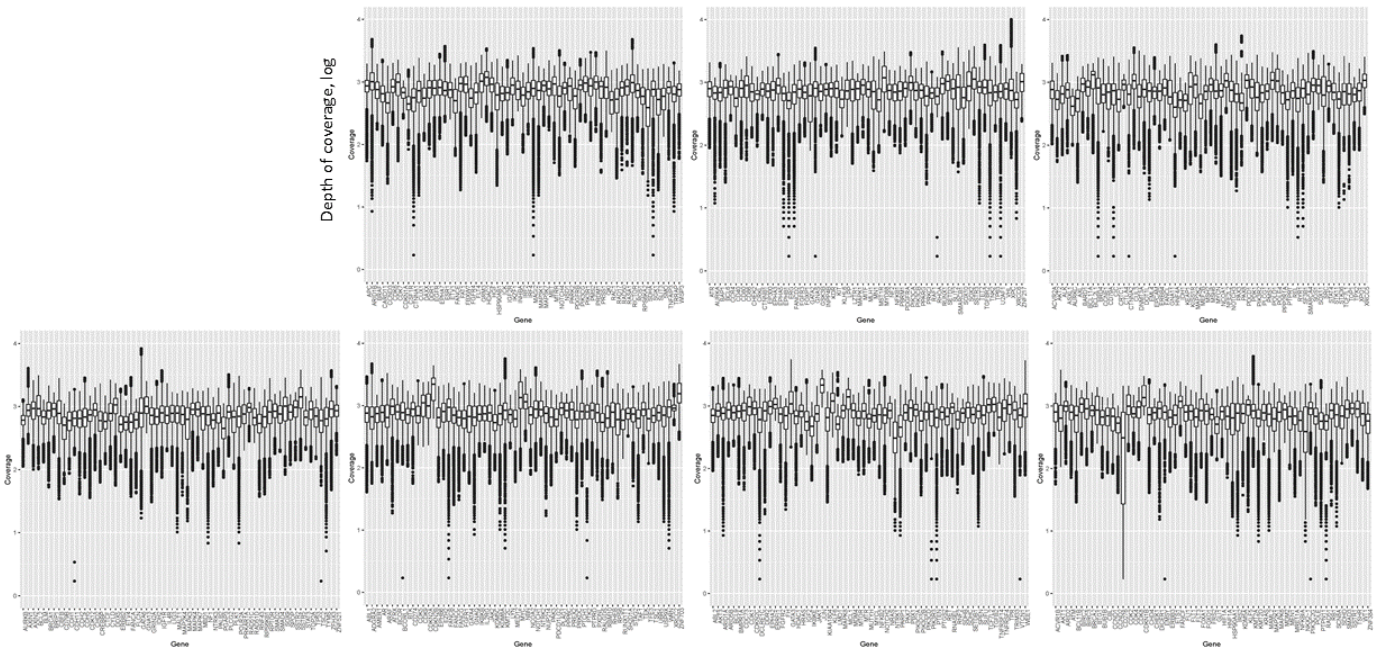
Supplemental Figure 2. Validation of sequence coverage depth across all 435 panel genes. 100 specimens underwent CANCERPLEX testing and the depth of coverage is shown on the y-axis of the box plot. Each of the 435 genes on the panel is shown on the x-axis. Open boxes represent mean coverage and lines show standard deviation. Outliers are depicted by black circles. Note that noncoding exons are included.

Supplemental Figure 3. **Validation of copy-number profiles generated by CANCERPLEX**. **(A)** Global CNV profile for cell line SNU-16 based on CANCERPLEX and visualized by CNVkit. **(B)** Comparison to Affymetrix SNP6.0 array visualized by IGV (SNU-16, CCLE data). **(C)** CNV profile for clinical case #1194. Focal *CDKN2A/CDKN2B* loss was detected (9p21.3). **(D)** P16 IHC staining, case #1194. Negative staining of glioblastoma tumor cells. Positive cell is residual normal neuron (internal positive control). **(E)** CNV profile for clinical case #1180. Focal amplification of *ERBB2 (HER2/neu)* was detected (17q12). **(F)** HER2/CEN17q dual-probe FISH assay for the CRC case #1180. HER2 signal (red) is amplified confirming CANCERPLEX findings.

Supplemental Figure 1.



Supplemental Figure 2.



Supplemental Figure 3.