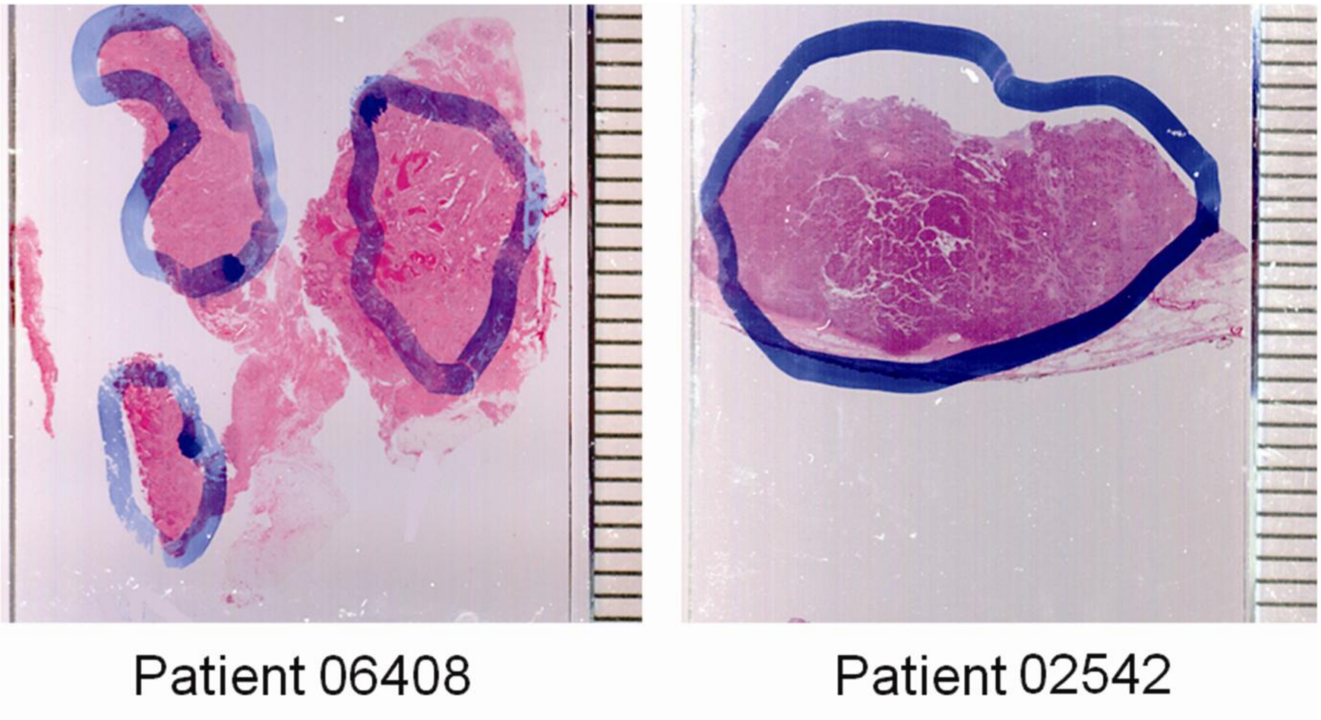
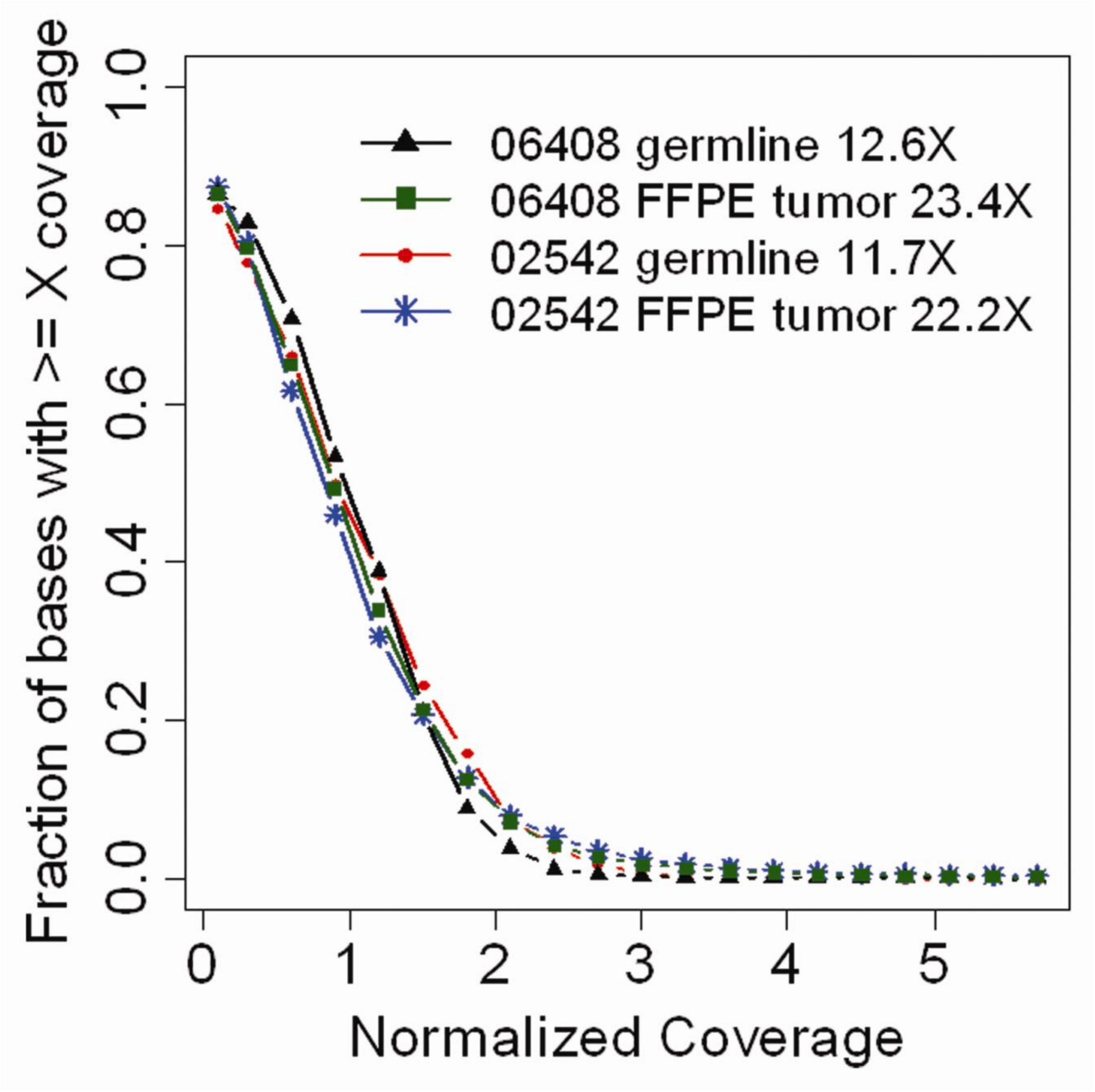
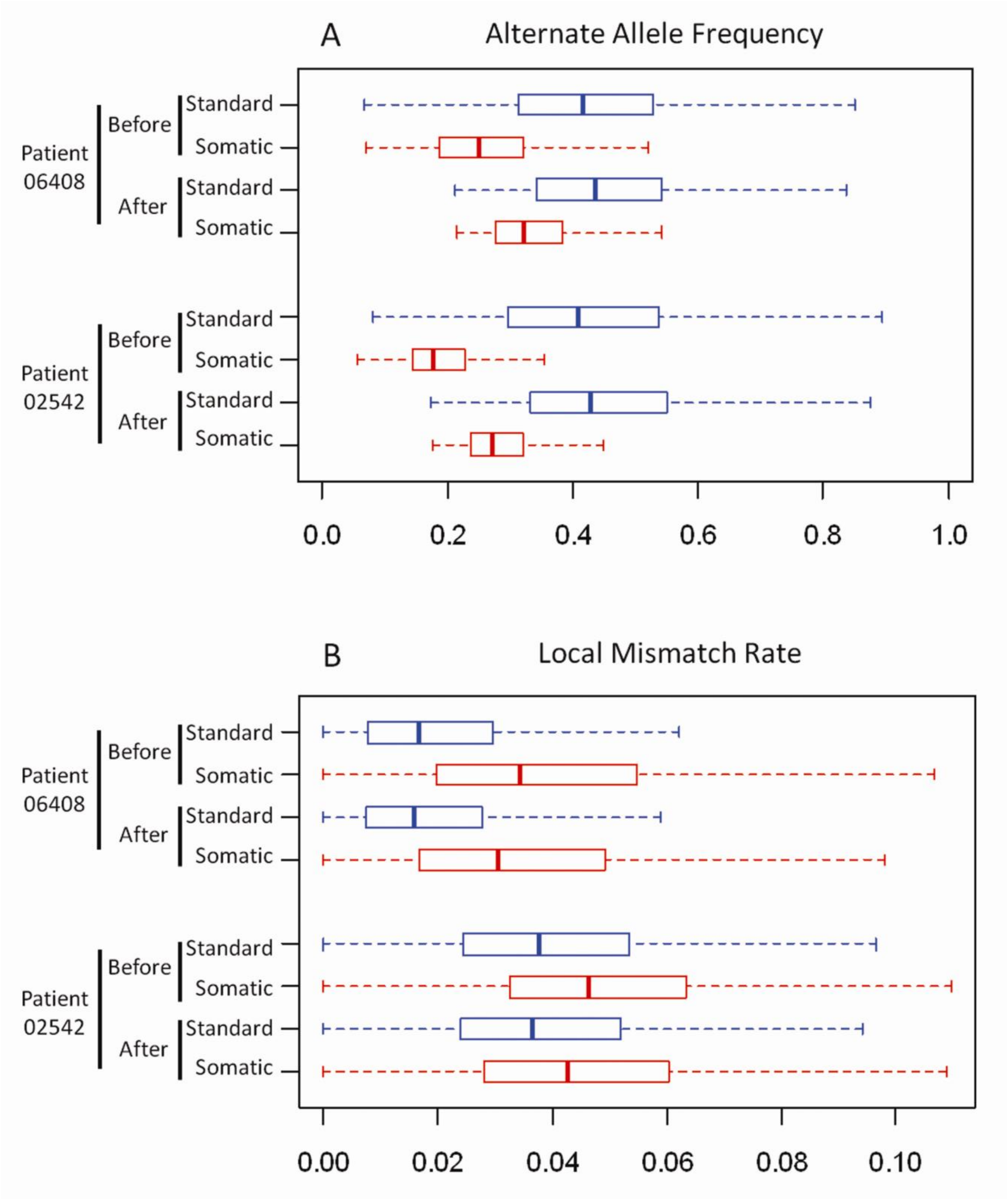


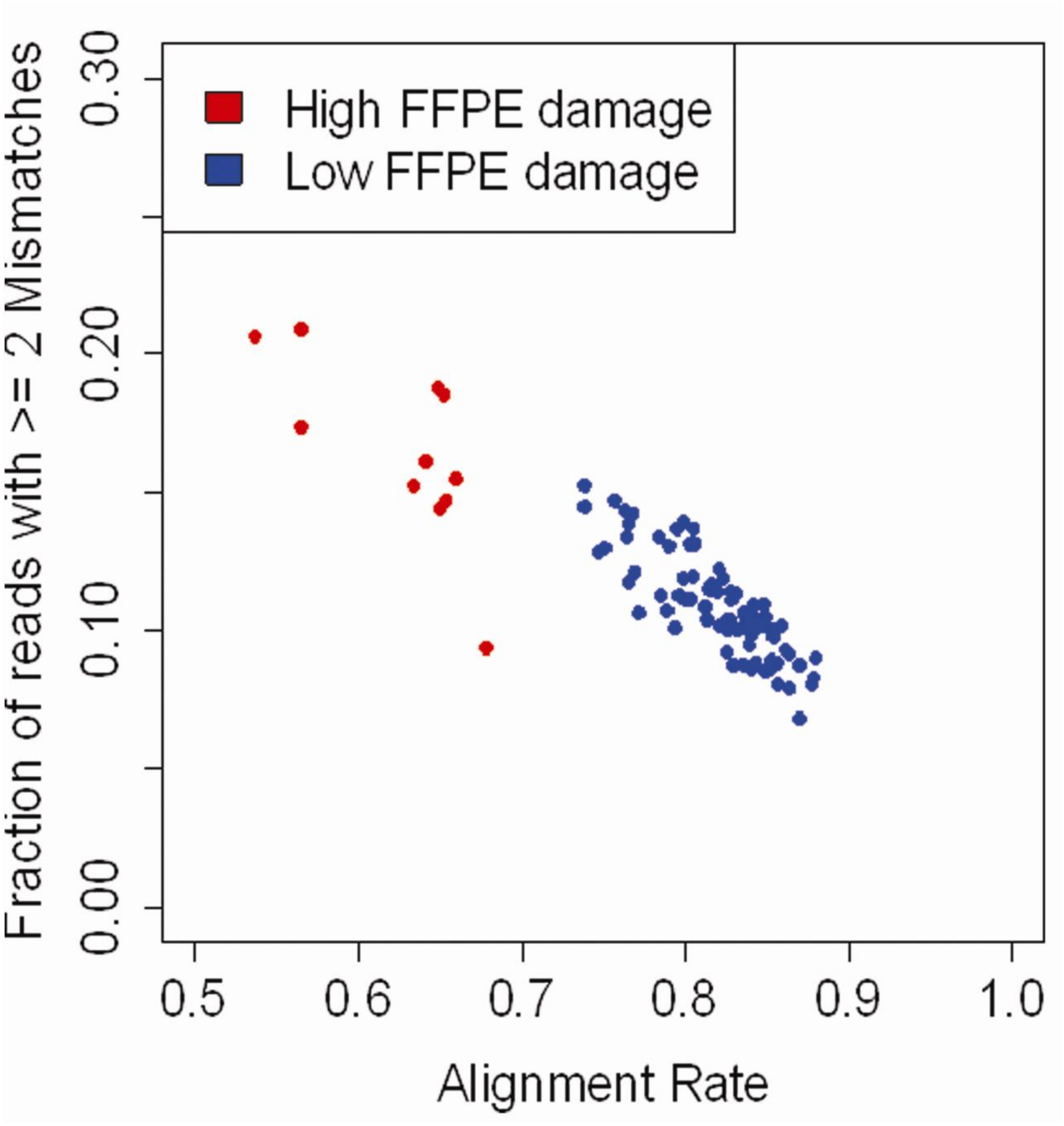
Supplemental Figure 1. Images of H&E stained 5 micron sections of FFPE tumor samples. The areas of high tumor density (80-85%) were marked by a pathologist and used as guidelines for isolating cells on the adjacent unstained slide for DNA isolation.



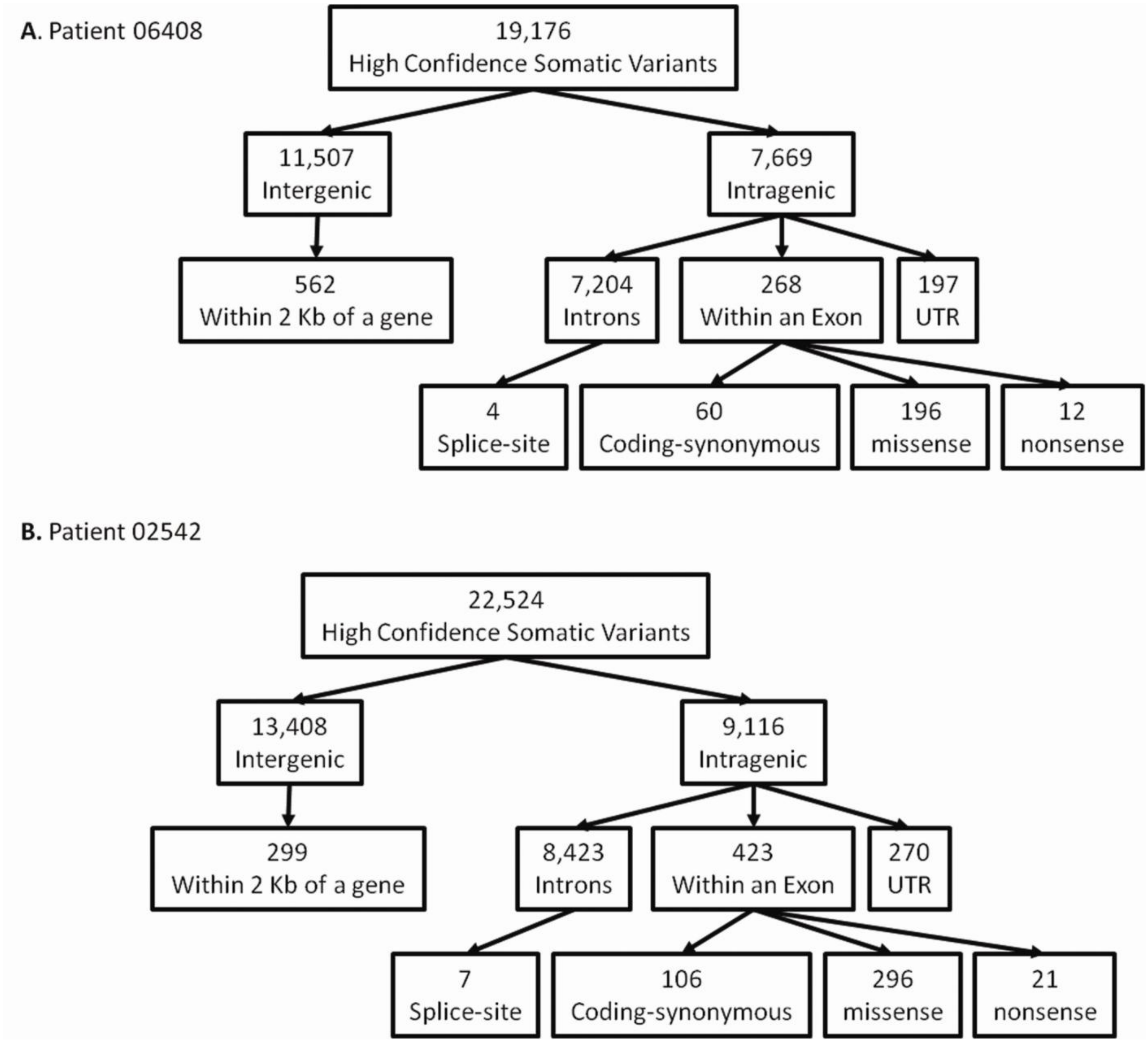
Supplemental Figure 2. Normalized genomic coverage distribution. The distributions for germline and FFPE tumor reads from patients 06408 and 02542, using non-duplicate reads after local realignment and excluding chromosome Y.



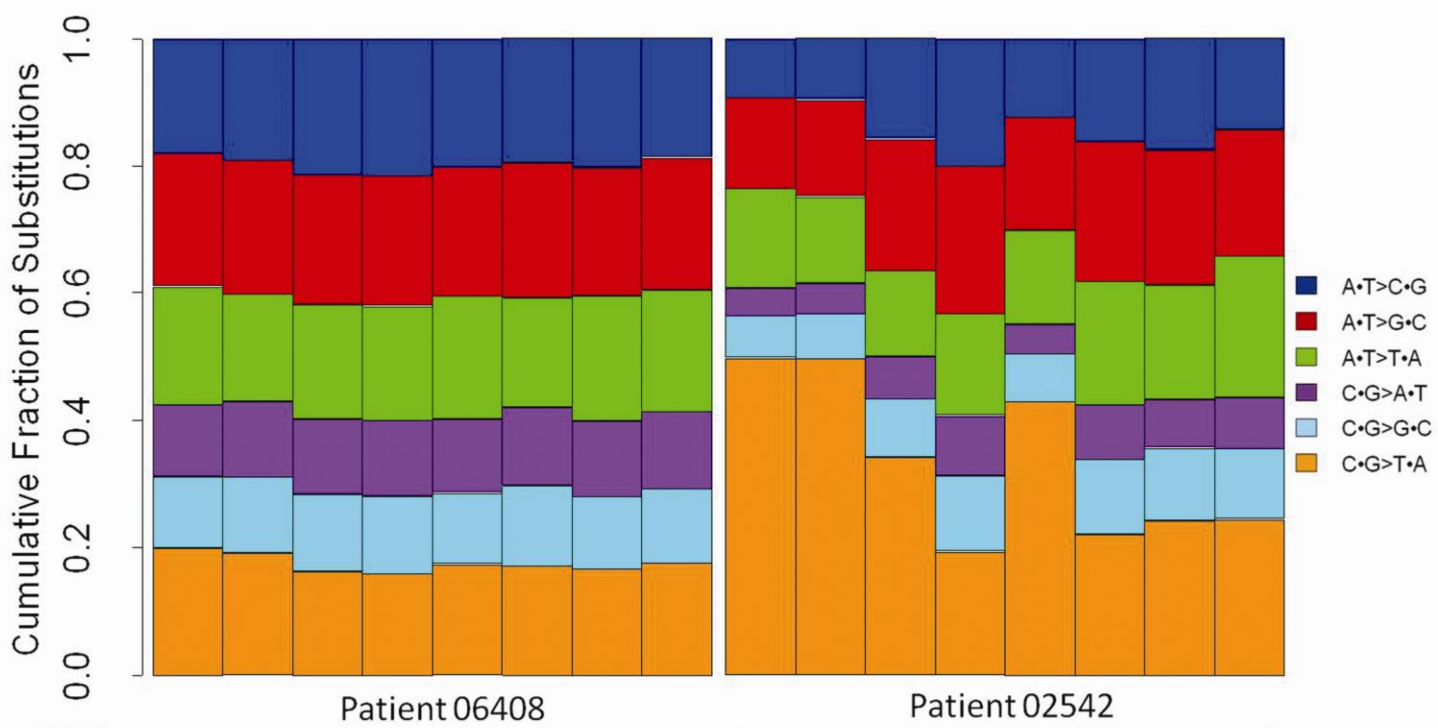
Supplemental Figure 3. Effect of Filter 2.5 (Remove variants in regions of high local mismatch rate) on **(A)** alternate allele read frequency and **(B)** local mismatch rates. Shown are the gold standard set of heterozygous variants (Standard) and candidate somatic variants (Somatic) 'Before' and 'After' Filter 2.5, for patients 06408 and 02542. Boxes are the 25 percentile and whiskers extend to 99.95 percentile.



Supplemental Figure 4. Analysis of Illumina sequencing reads of 89 FFPE tumors. A k-means clustering of the alignment rates and the fraction of aligned reads with ≥ 2 mismatches separated the samples into two groups indicated by the red and blue dots. Samples in the red group likely have a greater amount of FFPE DNA damage than the samples in the blue group.



Supplemental Figure 5. Annotation of high-confidence somatic variants for **A)** patient 06408 and **B)** patient 02542.



	Patient 06408								Patient 02542							
Somatic Mutations:	55,551	39,303	19,750	19,176	25,203	35,062	23,612	45,875	290,340	134,468	35,732	22,523	84,779	56,178	40,907	103,095
Filter 2.4:		X	X	X		X				X	X	X		X		
Filter 2.5:			X	X	X		X				X	X	X		X	
Filter 2.6:				X		X	X	X				X		X	X	X

Supplemental Figure 6. The inclusion of Filters 2.4, 2.5 and 2.6 are necessary to remove potential false positive somatic mutations. Shown are different combinations of Filters 2.4, 2.5 and 2.6. The top line shows the number of somatic mutations present after applying the indicated filters. The total number of candidate somatic mutations and the fraction of substitution types differ depending on the combinations of Filters 2.4, 2.5, and 2.6 applied to patient 06408 and 02542 FFPE tumors. All three filters are needed to obtain the appropriate number of somatic mutations and distribution of substitution types.