

## Introduction

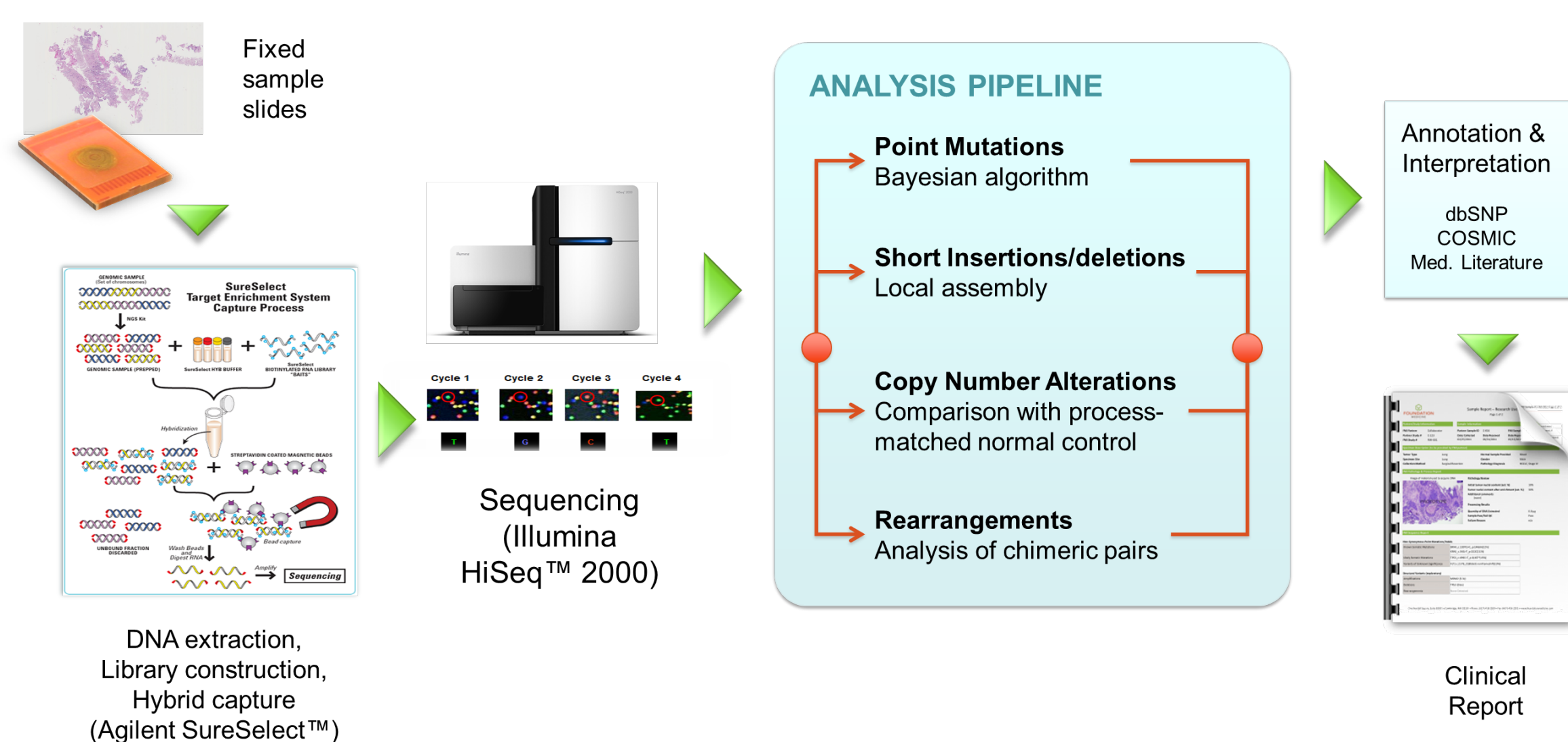
Comprehensive cancer genomic testing using Next-Generation Sequencing (NGS) technology identifies many more alterations than traditional methods. Some of these alterations, whether previously known or novel, may aid therapeutic decision making. To realize its full potential to help inform clinical cancer treatment, an NGS-based test must perform well on clinically relevant tissue specimens, principally formalin-fixed paraffin-embedded (FFPE) surgical resections, needle biopsies, or fine needle aspirates which may vary significantly in age. Furthermore, detection of somatic mutations in impure and heterogeneous tumor specimens requires high depth of coverage, in contrast to that of germline variants. This is challenging in that formalin fixation degrades nucleic acids, making molecular analyses challenging for such samples. Here we present a targeted NGS-based test that delivers high quality sequencing results from FFPE specimens representing four tissues of origin and ranging from one to eleven years old.

## Materials and Methods

DNA was isolated from three 20µm sections each from 96 FFPE specimens. Tissue specimens were either 1, 3, 5, 7, 9, or 11 years old, and for each year represented there were 2 tumor/normal pairs from each of the following tissue types: breast, colon, lung, and renal.

Indexed, ligation-based sequencing libraries were constructed using DNA extracted from each specimen. For specimens yielding >200ng after extraction, libraries were made using 200ng and (if enough DNA remained) 50ng of input DNA. For specimens yielding <200ng but >50ng of DNA, libraries were made using 50ng of input DNA, except in three cases where all of the extracted DNA (75-100ng) was used to make libraries. For specimens yielding <50ng of DNA, all of the extracted DNA was used as input into library construction.

Solution-based hybrid capture (custom Agilent SureSelect™) was used to enrich for the complete coding regions of 182 cancer-related genes, representing 1.1 Mb of target territory. The selected libraries were sequenced on an Illumina HiSeq™ 2000 platform using 49x49 paired-end reads.

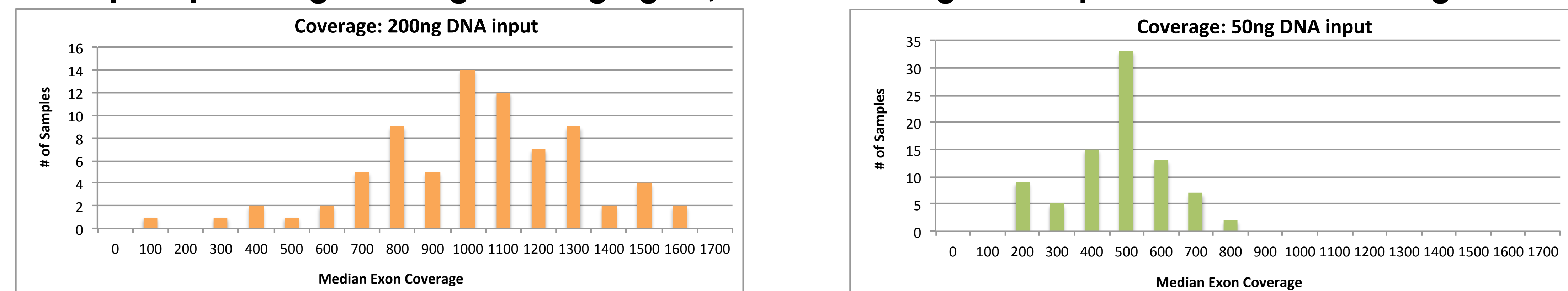


## Results

### High success rates throughout the process

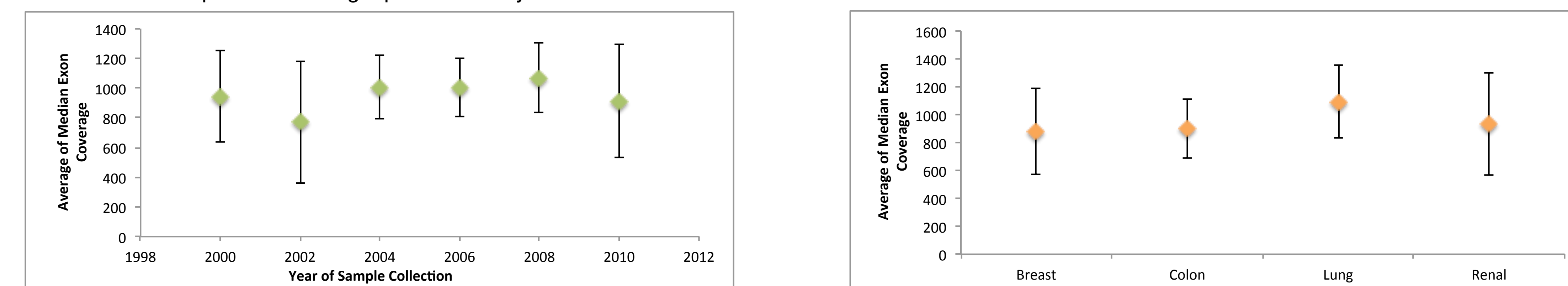


### Ultra-deep sequencing coverage averaging ~1,000x for 200ng DNA input and ~400x for 50ng DNA input

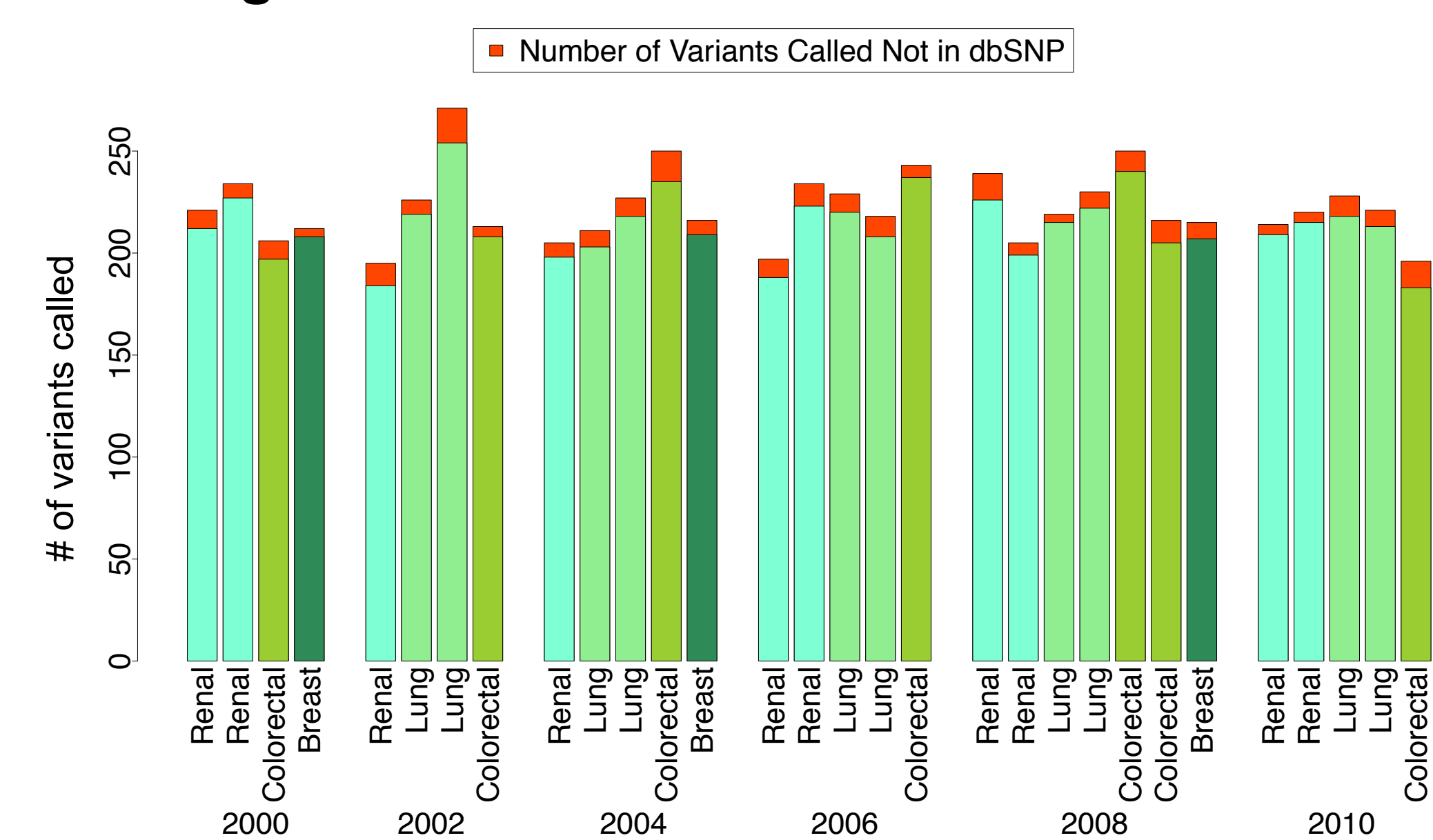


### Sequencing coverage is high regardless of tissue type or sample age

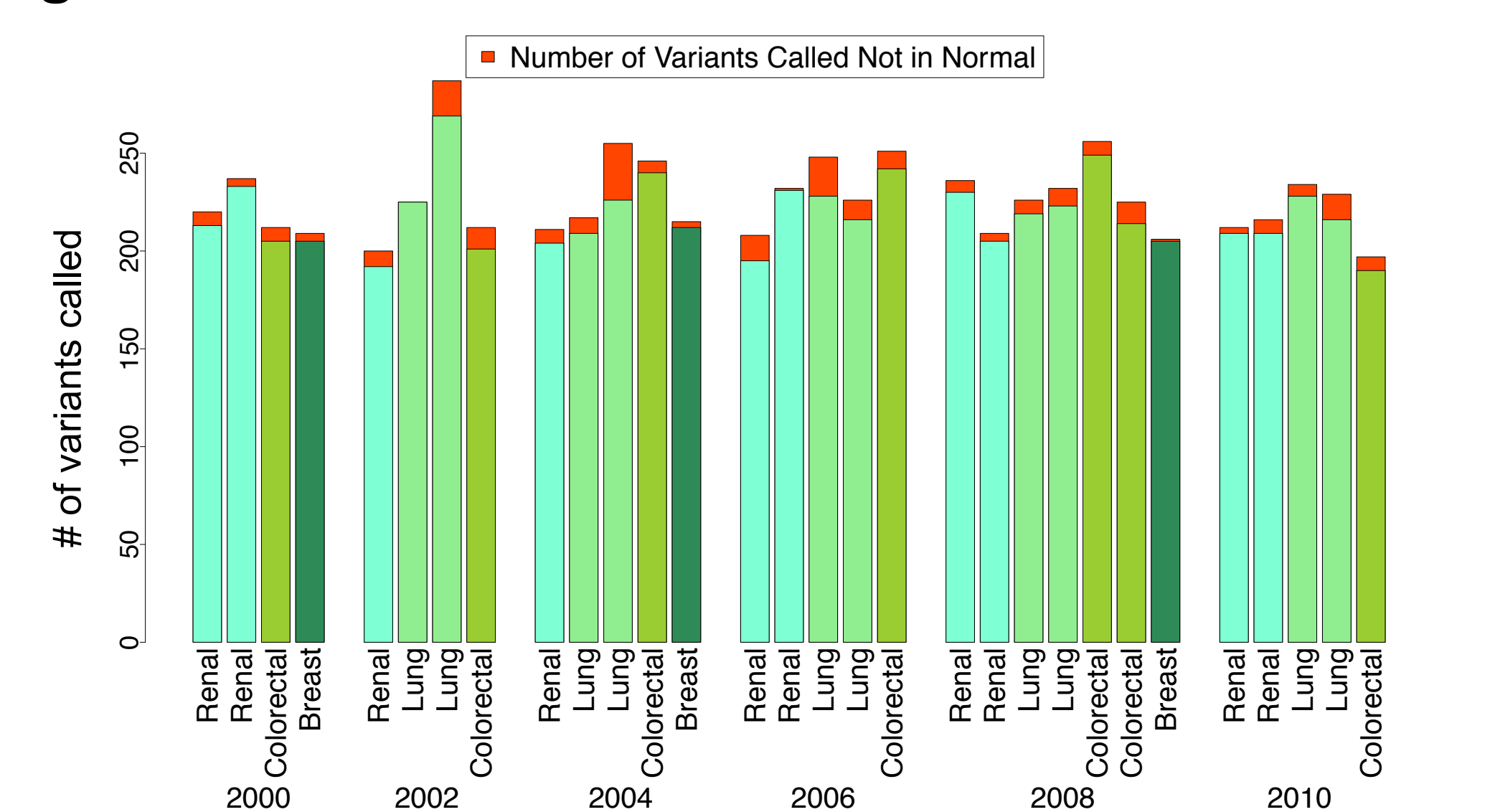
data shown for samples with 200ng input into library construction



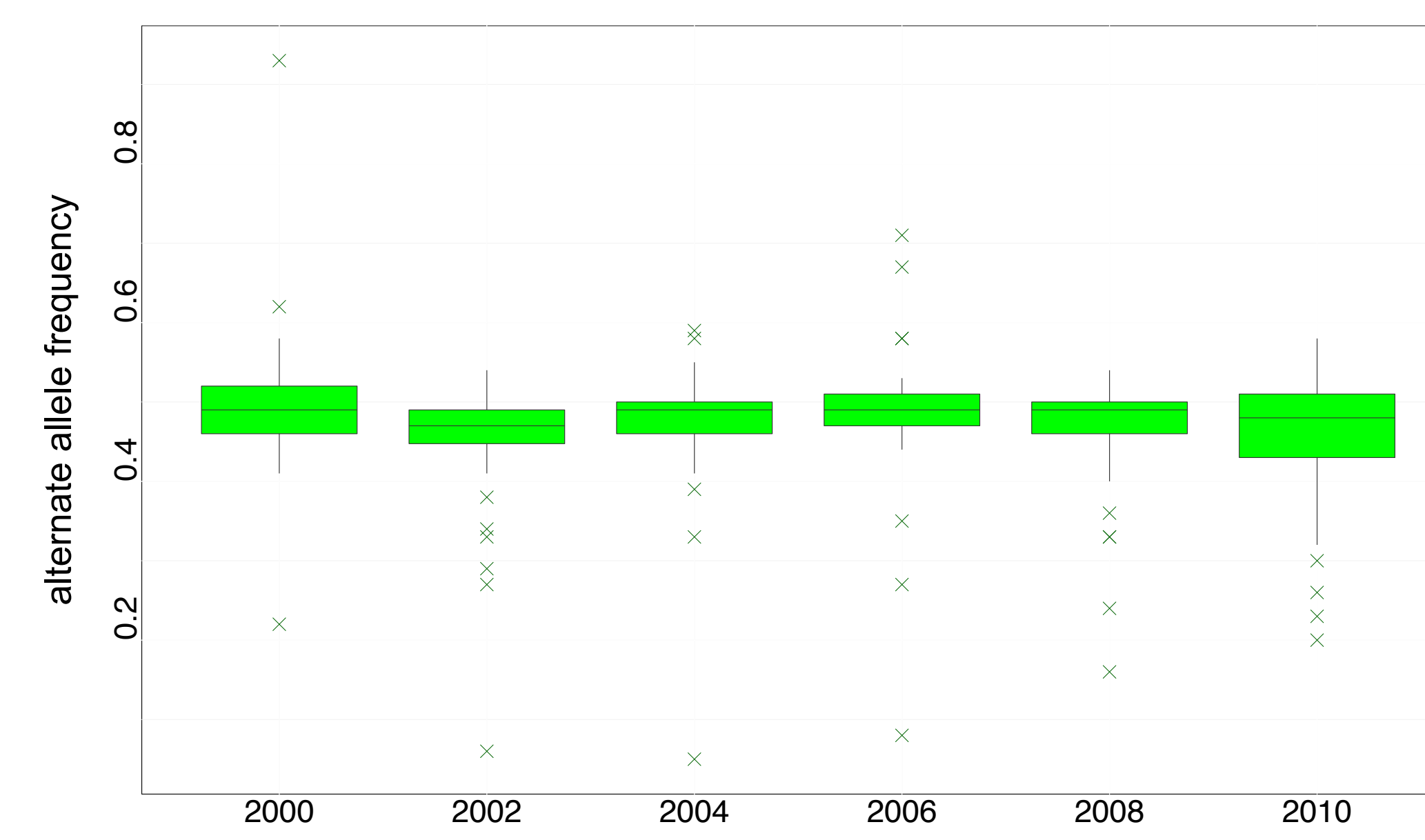
### In normal FFPE specimens, tissue type and sample age do not impact the # of variants called – nearly all are known germ-line variants



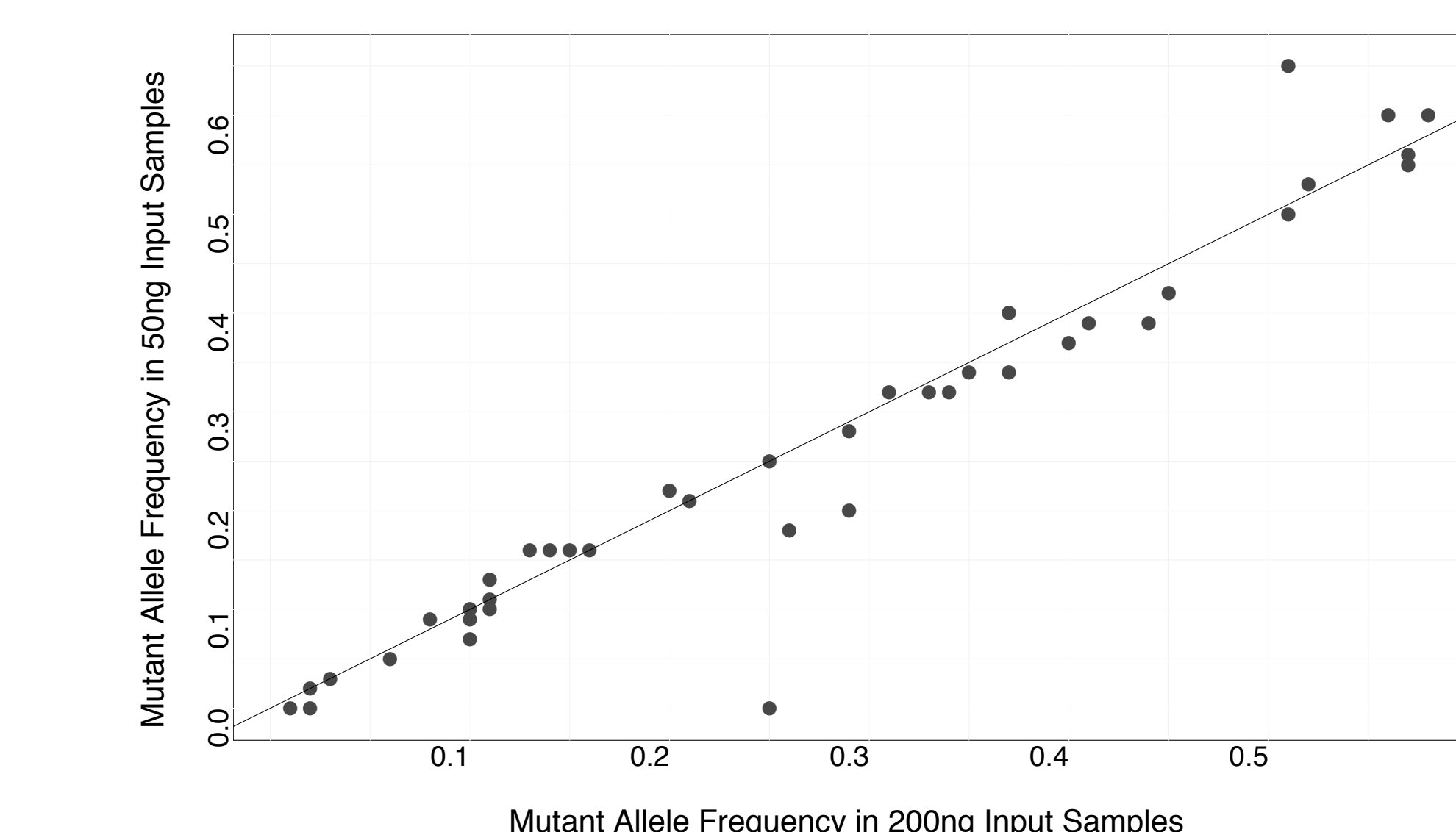
### In tumor FFPE specimens, tissue type and sample age do not impact the # of variants called – nearly all are germ-line variants also in matched normal control



### Novel variants discovered in normal FFPE specimens are most likely rarer SNPs

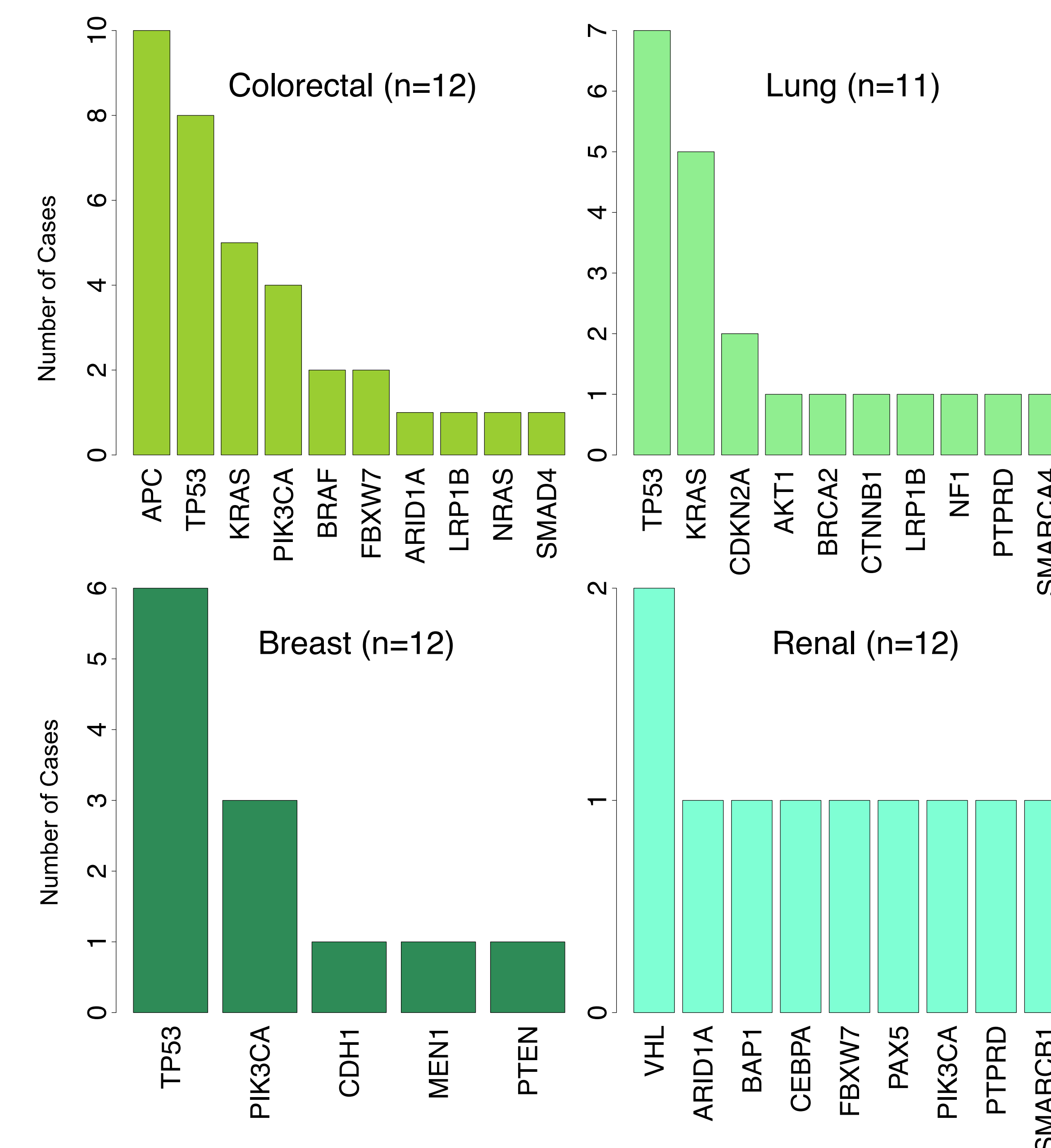


### Known somatic alterations called in 200ng input libraries of FFPE tumor specimens replicate in 50ng input libraries



## Somatic Alterations Detected

### A wide, tissue-appropriate spectrum of somatic alterations was detected



## Conclusions

- Ultra-deep (~1,000x) sequencing data can be routinely obtained from clinical FFPE specimens representing a wide range of ages and tissue types.
- The sequencing data from these FFPE specimens enables accurate mutation detection.
- These results indicate that comprehensive NGS-based testing should emerge as a routine part of cancer clinical trials and patient care.

## References

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