Gliomas are a common type of brain tumor accounting for ~80% of malignant brain tumors. Low-grade gliomas have better prognosis than high-grade gliomas but inevitably recur at higher grades, highlighting the need for improved understanding of molecular mechanisms of disease progression. Recent advances in molecular profiling of tumors have raised hopes for the development of targeted therapeutics based on specific genomic, epigenomic, and transcriptomic patterns. However, these efforts are complicated by the highly heterogeneous nature of glioma and the fact that tumor classification can depend on sampling strategy. Therefore, it is important to develop new tools to assess the full extent of tumor heterogeneity in a systematic and unbiased manner. Here we present an approach for deconstructing tumor heterogeneity using common laboratory equipment called Differential Gene Coexpression Analysis of Serial Sections (DGCASS). DGCASS combines a novel sampling strategy of serial cryo-sections with unbiased gene coexpression analysis to reveal patterns of gene activity that are shared or unique within tissue samples. We apply DGCASS to a single grade II oligoastrocytoma and identify groups of genes whose expression patterns are highly correlated within the tumor but not within normal human brain samples. In parallel, we employ a combination of exome sequencing, amplicon deep-sequencing, and droplet-digital PCR to identify and quantify the frequencies of somatic mutations over serial sections of the same tumor sample, revealing the existence of distinct clonal populations within the tumor. We show that specific clonal populations are associated with specific aberrant patterns of gene activity within the tumor. Our results indicate that DGCASS is an effective strategy for identifying the transcriptional consequences of specific mutations (or combinations thereof) within a single tissue sample, and suggest a simple and generalizable approach for deconstructing heterogeneity in other solid tumors.