Identification of LOH on chromosome arms 1p and 19q for molecular classification of gliomas with QIAseq® Targeted DNA Pro Panels and QIAGEN® CLC Genomics Workbench

Introduction

Molecular analyses have become part of routine disease classification, and adult-type diffuse gliomas serve as a prominent example of this. Loss of heterozygosity (LOH) or codeletion of chromosome arms 1p and 19q is characteristic of gliomas with oligodendroglial histology and is observed in up to approximately 80% of cases (1). This molecular event has been identified as a prognostic marker for affected patients with higher survival rates (2) and as a predictive marker for affected patients demonstrating elevated response rates (3). In addition, mutations in the *isocitrate dehydrogenase 1* (*IDH1*) gene have been identified in the majority of gliomas with the oligodendroglioma and astrocytoma histologic subtypes (4). To assess these molecular features, detailed analyses of specific genomic areas of interest are necessary. QIAseq Targeted DNA Pro Panels offer focused catalog panels as well as fully customizable designs, and, together, these facilitate cost-efficient and detailed analysis. The entire workflow for QIAseq Targeted DNA Pro Panels — from extracted DNA to sequencing-ready libraries — is completed within 6 hours. Extracted DNA is fragmented, genomic targets are molecularly barcoded and enriched, and libraries are constructed with an enzymatic cleanup after ligation and target enrichment. The QIAGEN CLC Genomics Workbench provides an easy-to-use graphical user interface for the analysis of a vast array of genomics data, including QIAseq Targeted DNA Pro data. Pre-configured template workflows enable calling of complex genomic alterations and visual inspection of results.

Here, we describe the identification of LOH on chromosome arms 1p and 19q in the oligodendroglioma-derived cell line BT88 using the Biomedical Genomics Analysis plugin of the QIAGEN CLC Genomics Workbench. Importantly, by diluting BT88 DNA with DNA from the NA24385 cell line, we show that the identification of LOH is feasible in a dilution representing a tumor purity of approximately 60%. In addition, we demonstrate detection of the *IDH1*:p.R132H mutation in the oligodendroglioma-derived BT54 cell line.

Data and analysis

DNA samples from the BT54 and BT88 cell lines were subjected to library preparation and target enrichment using the QIAseq Targeted DNA Pro workflow with 5 and 10 ng of input DNA, respectively. This protocol includes incorporation of unique molecular indices (UMIs) that allow a substantial reduction in PCR and sequencing errors and, hence, improves detection of genetic alterations. In addition, the use of UMIs facilitates estimation of the original number of DNA fragments used as input and,

thereby, reduces PCR amplification bias across genomic regions. The Comprehensive Cancer Research Panel (PHS-3000Z) catalog panel, which provides relative enrichment of chromosome arms 1p and 19q, was used for target enrichment. BT88 DNA was diluted with NA24385 DNA to obtain the following percentages of BT88 DNA: 100%, 80%, 60% and 40%. BT88 samples were sequenced on an Illumina® NextSeq® platform and BT54 samples were sequenced on an Illumina MiSeq®. All samples were subjected to paired-end sequencing using 300-cycle kits. The resulting data for all samples was analyzed with the "Identify QIAseq DNA Pro Somatic Variants with LOH Detection (Illumina)" template workflow available in the Biomedical Genomics Analysis plugin of the QIAGEN CLC Genomics Workbench. This template workflow is a pipeline of algorithms with preconfigured parameter settings for optimal handling of QIAseq Targeted DNA Pro Panel read structure, including UMIs, as well as components for trimming, mapping, realignment, variant calling, copy-number-variant detection, LOH estimation, filtering and annotation of the detected variants and quality control reporting. For LOH detection, the "Detect Regional Ploidy" tool uses coverage ratios from the "Copy Number Variant Detection" tool and variant allele frequencies of putative heterozygous germline variants to estimate the ploidy states. Variant allele frequencies, in the tumor-only type of setup used here, are estimated from somatic variant calls overlapping entries in a database of known segregating variants (e.g., dbSNP). The ploidy states "deletion" and "uniparental disomy" were considered to represent LOH. Chromosome-arm-level LOH was assessed using the sum of the length of chromosome arm LOH events relative to the size of the region covered from the first to the last target on the relevant chromosome arm. Somatic variants were detected by the "Low Frequency Variant Detection" tool and filtered in a comprehensive filtering cascade to remove variants that were likely to be false positives.

Results

1p and 19q loss of heterozygosity

The ploidy state was initially assessed for all target regions in the Comprehensive Cancer Research Panel. Adjacent targets demonstrating similar ploidy states were merged to represent events covering larger genomic regions. To estimate whether LOH events could be considered arm-level or focal, we defined a cutoff for relative chromosome-arm coverage at 2/3 for events to be considered arm-level. The expected coverage log2-ratios and b-allele frequencies (BAFs) for LOH events at the various tumor purities are outlined in Table 1.

Table 1. Coverage log2-ratios and b-allele frequencies expected for deletions and uniparental disomies at the sample purities created and analyzed in this study.

| Sample purity	Ploidy state	Coverage log2-ratio	BAF
100%	Deletion	-1.00	100%
	Uniparental disomy	0.00	100%
80%	Deletion	-0.74	83%
	Uniparental disomy	0.00	90%
60%	Deletion	-0.50	71%
	Uniparental disomy	0.00	80%
40%	Deletion	-0.32	63%
	Uniparental disomy	0.00	70%

BAF: b-allele frequency.

The results for all BT88 and NA24385 mixed samples are summarized in Table 2.

Table 2. Overview of detected LOH for the 1p and 19q chromosome arms in BT88 and NA24385 mixed samples.

BT88 fraction	1p LOH fraction	19q LOH fraction	Codetection of 1p19q LOH
100%	1.00	1.00	+
80%	0.89	0.91	+
60%	1.00	0.89	+
40%	0.30	0.22	-
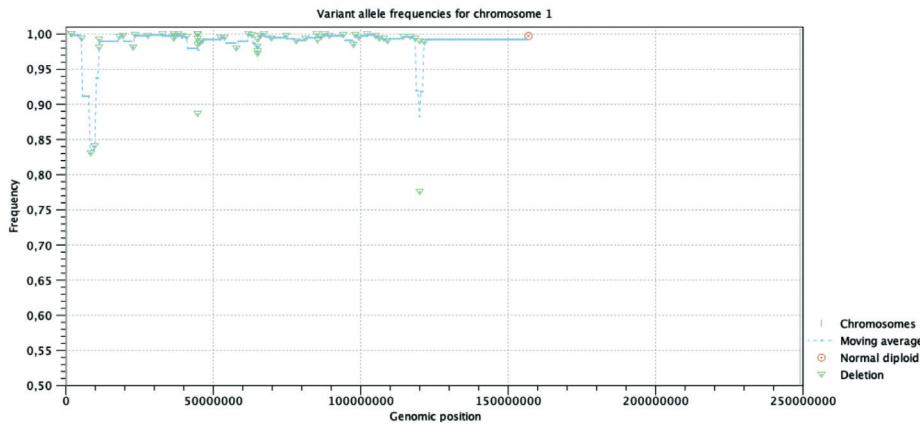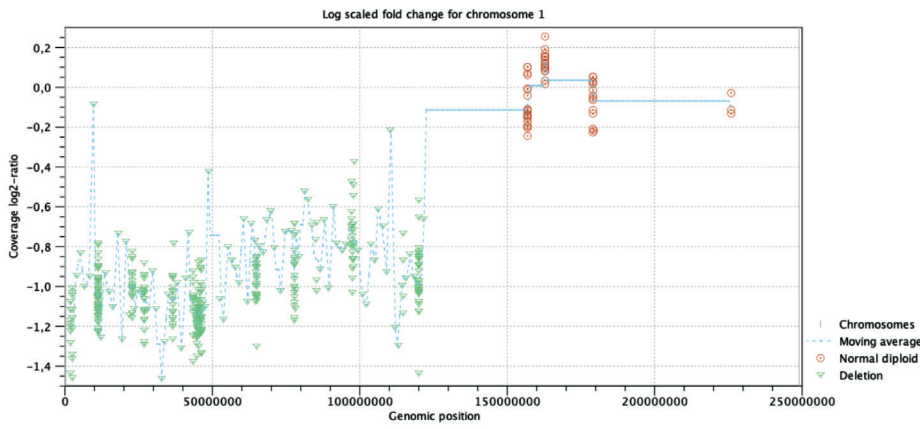
Figure 1. Coverage log2-ratios (top panel) and b-allele frequencies (bottom panel) across chromosome 1 for the 100% BT88 sample identified with the "Identify QIAseq DNA Pro Somatic Variants with LOH Detection (Illumina)" workflow and present in the "Regional_ploidy_results_report".
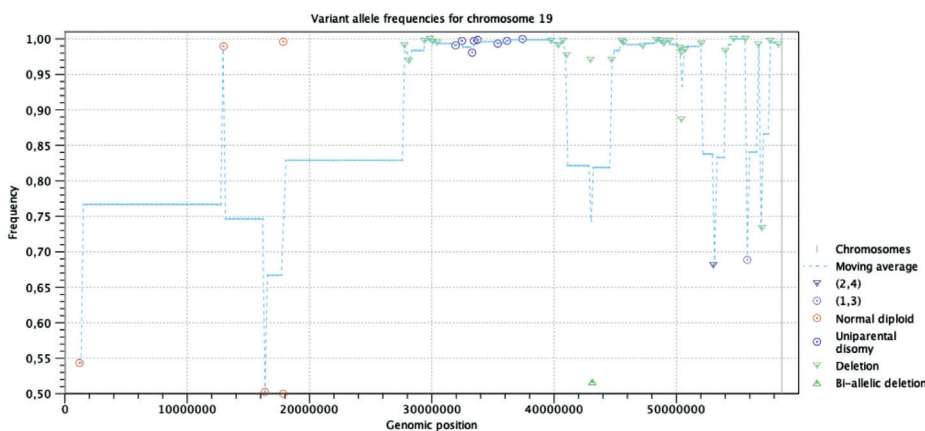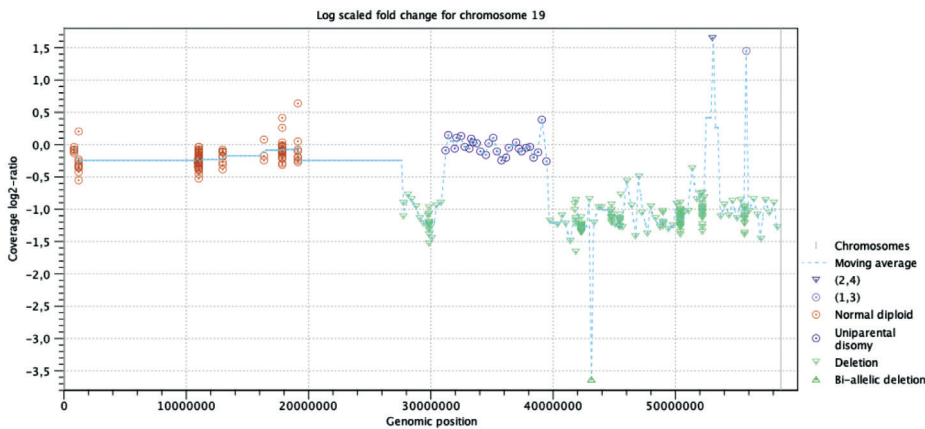
Figure 2. Coverage log2-ratios (top panel) and b-allele frequencies (bottom panel) across chromosome 19 for the 100% BT88 sample identified with the "Identify QIAseq DNA Pro Somatic Variants with LOH Detection (Illumina)" workflow and present in the "Regional_ploidy_results_report".

The QIAGEN CLC Genomics Workbench offers extensive visualization that makes it possible to inspect the details of the estimated ploidy states. Assessment of the coverage log2-ratios and BAFs underlying the estimated ploidy states for the 100% BT88 sample revealed a deletion of chromosome arm 1p (Figure 1) and a mixture of deletion and uniparental disomy for chromosome arm 19q (Figure 2).

For diluted BT88 samples, noise resulting from uneven representation of BT88 and NA24385 DNA was expected to be introduced to both the coverage log2-ratio and the BAFs. Nevertheless, we detected LOH events that were in agreement with the baseline 100% BT88 sample for dilutions down to 60% sample purity (Figures 3 and 4). We do, however, note that more noise has been

introduced, as evidenced by a larger spread for coverage log2-ratios and BAFs and minor regions demonstrating different ploidy.

We recommend detection of LOH events on chromosome arms 1p and 19q to be performed with any of the following QIAseq Targeted DNA Pro catalog panels using the "Identify QIAseq DNA Pro Somatic Variants with LOH Detection (Illumina)" template workflow:

- PHS-004Z Brain Cancer Research Panel
- PHS-104Z Brain Cancer Focus Panel
- PHS-3000Z Comprehensive Cancer Research Panel
- PHS-3100Z Comprehensive Cancer Focus Panel
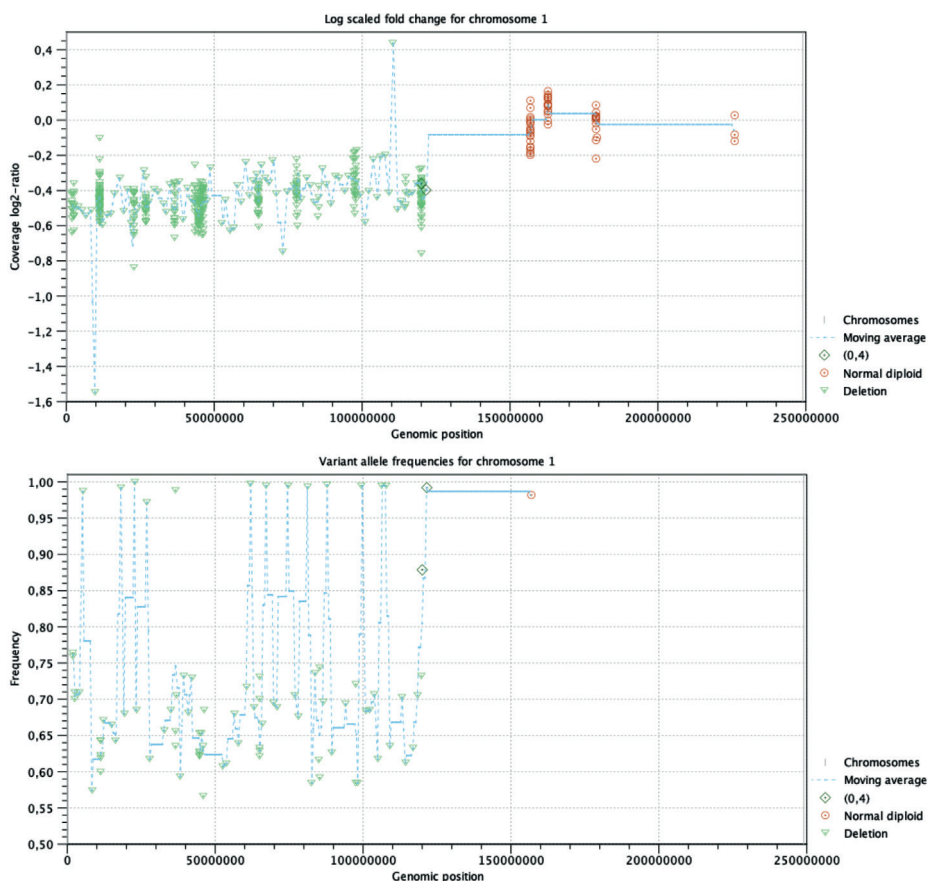
Figure 3. Coverage log2-ratios (top panel) and b-allele frequencies (bottom panel) across chromosome 1 for the 60% BT88 sample identified with the "Identify QIAseq DNA Pro Somatic Variants with LOH Detection (Illumina)" workflow and present in the "Regional_ploidy_results_report".

These panels are designed to provide relative enrichment of chromosome arms 1p and 19q, as outlined in Table 3.

Validation of LOH detection across the recommended panels was performed by reanalyzing the BT88 and NA24385 mixed samples and restricting the data processing of the template workflow to the sets of target regions available in each catalog panel. LOH events detected at the region level were summarized for each chromosome arm and normalized as described above. The fraction of 1p and 19q chromosome arms with LOH was similar across all four analyzed catalog panels for samples consisting of 60%, 80% or 100% BT88 (Figure 5).

Table 3. The number of targets covering regions on chromosome arms 1p or 19 and their relative contribution to the various catalog panels recommended for detection of LOH on chromosome arms 1p and 19q.

Catalog panel	Targets on 1p	Fraction of targets on 1p	Targets on 19q	Fraction of targets on 19q
PHS-004Z	189	0.18	109	0.11
PHS-104Z	88	0.33	88	0.33
PHS-3000Z	161	0.12	106	0.08
PHS-3100Z	405	0.09	206	0.04
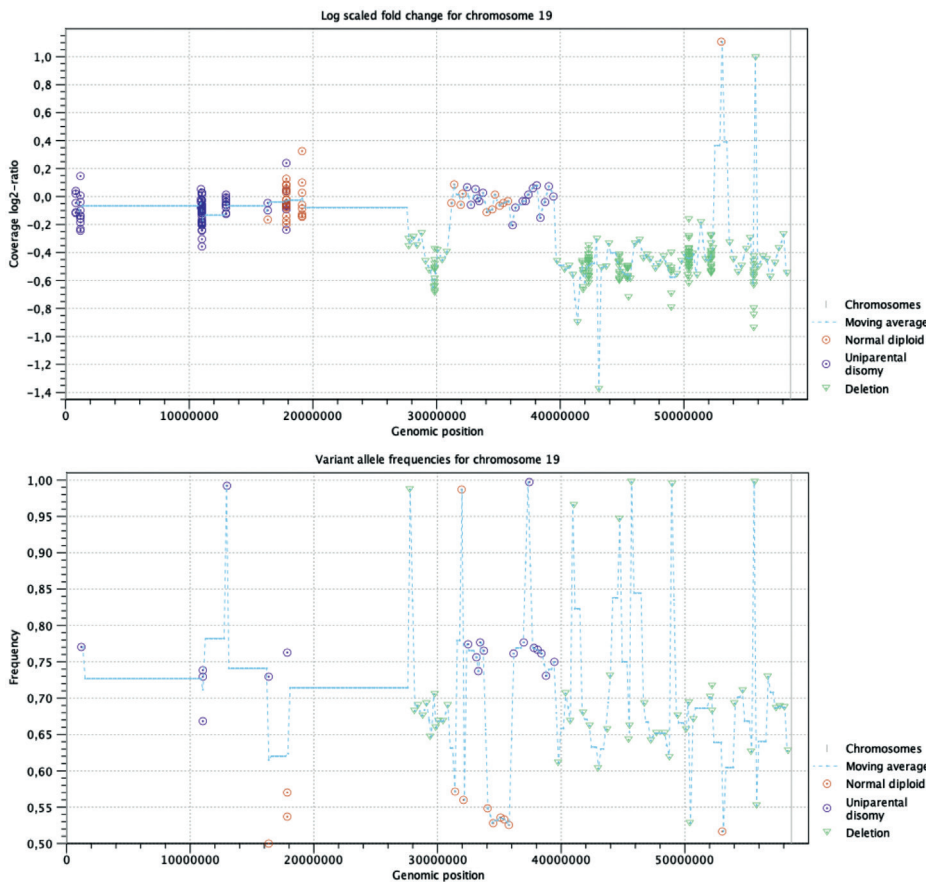
Figure 4. Coverage log2-ratios (top panel) and b-allele frequencies (bottom panel) across chromosome 19 for the 60% BT88 sample identified with the "Identify QIAseq DNA Pro Somatic Variants with LOH Detection (Illumina)" workflow and present in the "Regional_ploidy_results_report".

IDH1 mutation status

IDH1 mutation status represents a key component of the molecular classification of gliomas.

The recommended catalog panels for 1p and 19q LOH detection all enrich for the *IDH1* hotspot mutation at residue 132, which accounts for the vast majority of *IDH1* mutations (5). In addition, the Comprehensive Cancer Research Panel, Comprehensive Cancer Focus Panel and Brain Cancer Research Panel catalog panels also enrich for regions in *IDH2*, and the Comprehensive Cancer Research Panel and Brain Cancer Research Panel further enrich for additional *IDH1* regions. The *IDH1*-mutant oligodendroglioma-derived BT54 cell line was analyzed to verify detection of the *IDH1* hotspot mutation, as this mutation is not present in BT88 (6). The *IDH1*:p.R132H mutation was successfully identified in both of two replicates, as exemplified in Figure 6.
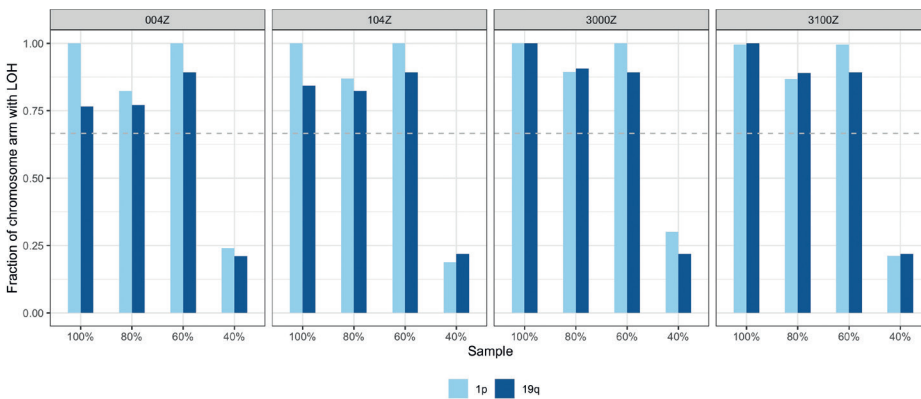

Figure 5. Detection of chromosome-arm-level LOH for BT88 and NA24385 mixed samples processed using the "Identify QIAseq DNA Pro Somatic Variants with LOH Detection (Illumina)" template workflow restricted to the 1p and 19q target regions. The grey dashed line represents the defined cutoff for chromosome-arm-level LOH at 2/3.
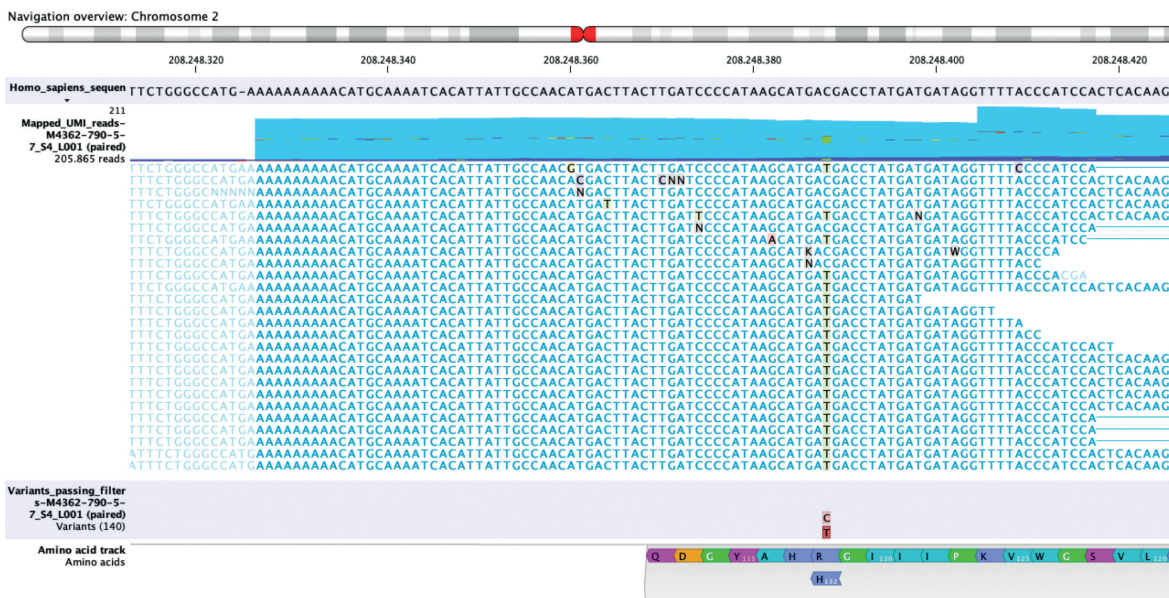

Figure 6. Representative overview of a *IDH1*:p.R132H (c.395G>A) mutation in the BT54 cell line presented in the QIAGEN CLC Genomics Workbench. The panels outlined are (from the top): the reference sequence, a read mapping including coverage graph, a variant track and an amino acid track.

Conclusion

Analysis of molecular events in clinical practice requires flexibility for responding to variation in tumor purity and in DNA input amount. Using the QIAseq Targeted DNA Pro workflow and the Biomedical Genomics Analysis plugin for the QIAGEN CLC Genomics Workbench, we demonstrate detection of LOH for 1p and 19q at tumor purities as low as 60% with a DNA input of only 10 ng.

Furthermore, we demonstrate identification of the *IDH1*:p.R132H mutation using the same sample processing and analysis setup. The combined assessment of *IDH1* mutation status and LOH or codeletion for 1p and 19q is important for molecular classification of gliomas, and we show the feasibility of this combined assessment with minimal sample input.

References

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