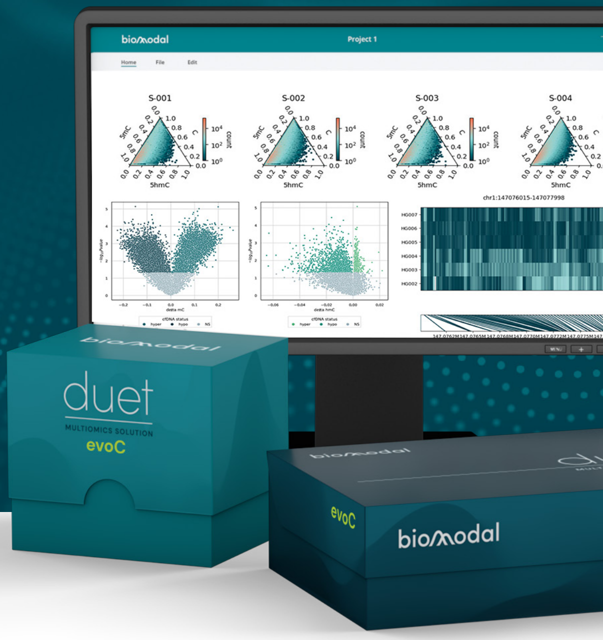
biomodal | MLL

CASE STUDY

# Large Diagnostic Laboratory Adopts 6-Base Sequencing To Improve Disease Characterisation



## Overview

Epigenetic patterns can serve as particularly valuable biomarkers when characterising disease. A growing body of evidence suggests that the distribution of both 5-methylcytosine (5mC) and 5-hydroxy methylcytosine (5hmC) changes in the early stages of disease development and may form disease-specific patterns. As such, many laboratories are interested in leveraging methylation sequencing technology to improve early disease detection and refine disease profiling.

The MLL (Munich Leukemia Laboratory) is one such facility. Their goal is to develop workflows that enable the detection of epigenetic patterns in leukemia patients, potentially enhancing diagnostic precision and patient stratification. The innovation arm of MLL's R&D department has investigated various methylation sequencing technologies and has selected biomodal's duet evoC 6-base sequencing technology as a top candidate for integration. Despite being early in their journey, MLL's team has praised the technology for its simplicity, uniformity, and depth of sequencing coverage, making it a promising tool for enhancing patient stratification and biomarker discovery.

## Early Results with duet evoC at MLL

- Uniform genomic coverage
- Deep sequencing depth
- Minimal background noise
- Exceptional ease-of-use of the workflow, consistently praised by experienced laboratory professionals

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>
> Wencke Walter, Head of Innovation at MLL

## The Path To Methylation Sequencing

Established in 2005, the MLL serves clinicians throughout Germany and Austria by providing diagnostic testing for various blood cancers. The laboratory's comprehensive capabilities span cytomorphology, cytogenetics, immunophenotyping, FISH, and molecular genetics (among many other testing modalities). To keep up with the field's evolving needs, an R&D team at MLL is consistently searching for new technologies and workflows that can bring unique diagnostic value to the lab.

Wencke Walter, Head of Innovation at MLL, emphasized the importance of this ongoing technological pursuit: "We are always looking for technologies that expand the amount of information we gain—particularly if it could lead to new biomarkers or enhance patient stratification."

To be integrated at MLL, new technologies must be highly accurate, efficient, and capable of providing maximal information in a minimal amount of time. When dealing with aggressive cancers such as acute myeloid leukemia (AML), rapid results turnaround time is critical.

While MLL has extensively used whole genome sequencing (WGS) and transcriptome sequencing (WTS), these approaches haven't always provided additional diagnostic value. Additionally, the team suspected that WGS and WTS were overlooking a critical part of disease pathology. "Whether from aging or other factors, patients with leukemia often have mutations that don't correlate well to effects in the transcriptome," explained Walter. Many of these mutations occurred in or near epigenetic regulators, prompting her team to take an interest in measuring the epigenome.

## The Challenge

MLL's initial attempts at methylation sequencing—which included enzymatic and bisulfite sequencing workflows—presented significant practical challenges. These methods required lengthy, hands-on processes that proved inefficient. This was amplified by poor adapter ligation, which consistently resulted in low library yields, increased sequencing costs, and limited throughput.

"The enzymatic sequencing was a little bit of a problem because the performance wasn't what we expected," Explained Walter. "The yield that we generated per library was lower than we had hoped for and therefore to get the results we wanted, we needed to use more sequencing resources than we had planned for." To integrate methylation sequencing into the laboratories offering, the team would need a more efficient and informative assay than traditional methylation sequencing technologies could provide.

## The Solution

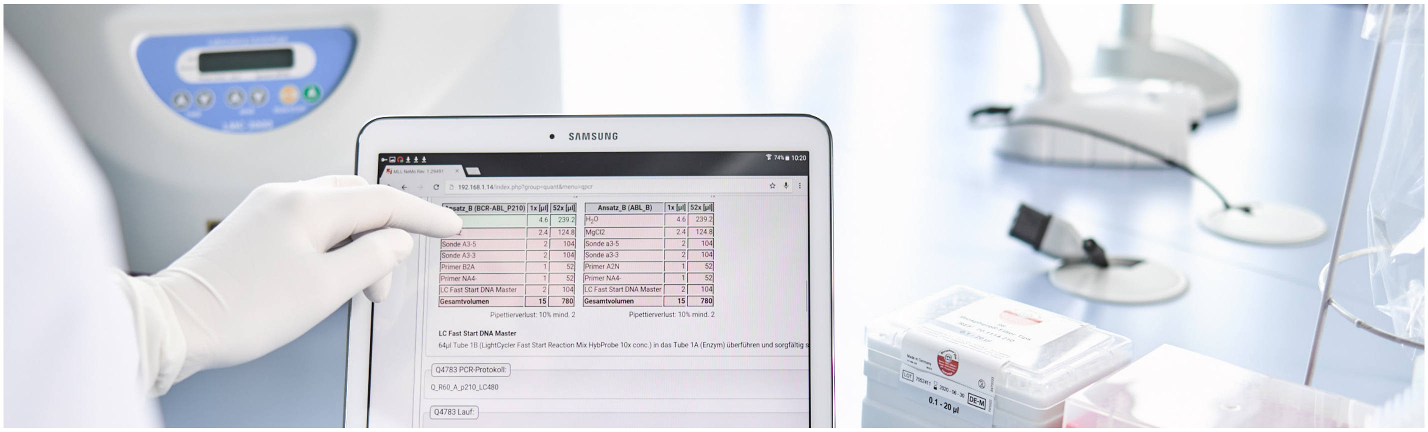
MLL turned to biomodal's duet evoC, a pioneering 6-base sequencing technology uniquely capable of distinguishing traditional DNA bases (A, T, C, G) along with two modified cytosines—5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC). This comprehensive sequencing solution resolves DNA methylation patterns with higher resolution, enabling better annotation of the epigenetic landscape and the subtle ways it may change during disease development.

Of particular interest to the team was duet evoC's ability to simultaneously provide both genomic and methylation data from a single sequencing run. According to Walter, the team saw in the technology the chance to "improve disease subtyping, especially for myeloid neoplasms, by integrating data from both methylation patterns and genomic variants."

## Method

The MLL team has begun preliminary testing of biomodal's duet evoC technology using 48 peripheral blood and bone marrow samples, materials that typically yield high-quality DNA suitable for next-generation sequencing (NGS). The samples underwent duet evoC's streamlined library preparation and sequencing protocol (notably, the duet evoC workflow is compatible with several commonly used NGS platforms).

The workflow simplicity stood out clearly, even among seasoned professionals accustomed to various NGS kits. Walter shared that, "Our team performs NGS library prep daily with multiple kits, and rarely do they specifically compliment a workflow. But while working with biomodal's solution, the team frequently commented on its ease of use and clearly described protocols."



| satz_B (BCR-ABL_P210) | 1x [µl] | 52x [µl] |
|---|---|---|
| [illegible] | 4.6 | 239.2 |
| [illegible] | 2.4 | 124.8 |
| Sonde A3-5 | 2 | 104 |
| Sonde A3-3 | 2 | 104 |
| Primer B2A | 1 | 52 |
| Primer NA4- | 1 | 52 |
| LC Fast Start DNA Master | 2 | 104 |
| **Gesamtvolumen** | **15** | **780** |

Pipettierverlust: 10% mind. 2

| Ansatz_B (ABL_B) | 1x [µl] | 52x [µl] |
|---|---|---|
| $H_2O$ | 4.6 | 239.2 |
| MgCl2 | 2.4 | 124.8 |
| Sonde a3-5 | 2 | 104 |
| Sonde a3-3 | 2 | 104 |
| Primer A2N | 1 | 52 |
| Primer NA4- | 1 | 52 |
| LC Fast Start DNA Master | 2 | 104 |
| **Gesamtvolumen** | **15** | **780** |

Pipettierverlust: 10% mind. 2

**LC Fast Start DNA Master**

64µl Tube 1B (LightCycler Fast Start Reaction Mix HybProbe 10x conc.) in das Tube 1A (Enzym) überführen und sorgfältig s

Q4783 PCR-Protokoll:

Q_R60_A_p210_LC480

Q4783 Lauf:

## Results

Early experiments with biomodal's duet evoC technology yielded highly encouraging outcomes. The protocol delivered uniform and deep genomic coverage with notably low background noise—critical factors for accurate methylation analysis. Walter expressed satisfaction, saying, "We have much higher output than we initially anticipated, so this technology is definitely an improvement. Plus, the sequencing was exceptionally good—the coverage looked very good, and the noise was quite low."

Anyone who has performed next generation sequencing knows that the wet-lab work is only part of the battle. As a trained bioinformatician, Walter knows that processing the resulting data can be a significant bottleneck for many laboratorians. "The data processing pipeline that's provided with biomodal's technology does 90% of the work," she explained. "That allows us to focus more on the harder part of any multiomic data set—interpretation. But, from a purely computational side, processing of the duet evoC data is very nice and easy."

Though MLL remains at the early stages of implementing duet evoC, their preliminary experiences have generated significant enthusiasm. The simplified and efficient workflow, paired with high-quality sequencing data, positions the technology as a pivotal tool in their evolving diagnostic arsenal.

This early success has inspired the team to move past the basic validation of the technology's quality and begin asking biologically meaningful questions with it. Their goal with the technology is clear: identifying robust, clinically relevant methylation patterns that improve patient stratification and potentially point to clinically valuable biomarkers.

## Future Directions

Looking ahead, MLL aims to further validate and integrate biomodal's 6-base sequencing technology into larger, more comprehensive multimodal analyses. They plan to investigate targeted sequencing approaches and systematically evaluate differential methylation linked to specific mutations, potentially enhancing diagnostic precision and patient care.

Walter summarizes MLL's outlook succinctly: "We are excited to see how far duet evoC can take us toward improving diagnostics and ultimately patient outcomes."