

CeGaT's technicians perform automated NGS library preparation on Hamilton STARs

Automating NGS Library Preparation for TMB and MSI Analysis

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Summary

CeGaT is a diagnostics company, highly active in the oncology field. Due to the high sample throughput for the analyses of Tumor Mutational Burden (TMB) and Microsatellite Instability (MSI), CeGaT decided to automate two of the next-generation sequencing (NGS) library preparation workflows used for this purpose. The Hamilton Microlab® STAR™ platform provided CeGaT with the best solution; a highly customizable deck with intuitive and flexible software that allowed them to easily program and update workflows on their own.

About CeGaT

Founded in Tübingen Germany in 2009, CeGaT is a privately-owned diagnostics company offering services for molecular analysis in the fields of medical diagnostics (Patient Care), basic research & clinical research (Pharma). CeGaT's laboratory is accredited as a human genetic diagnostic laboratory by the College of American Pathologists (CAP), the Clinical Laboratory Improvements Amendments (CLIA) and the International Organization for Standardization (ISO) 15189, thus meeting the highest international standards.

[Learn more about the company](#)



Medical Relevance of TMB and MSI Analysis

CeGaT is particularly active in the area of Precision Medicine (PM) Oncology, where it offers various standard and customized services for Next-Generation Sequencing (NGS). The company is also involved in several clinical research projects in this area (Table I).

Two of the most popular analyses in the field of PM-Oncology are Tumor Mutational Burden (TMB) and Microsatellite Instability (MSI). TMB refers to the number of mutations present in a patient's tumor (but not the corresponding healthy tissue). TMB is quantified as mutations per million base pairs (mut/Mbp) and classified as "low", "intermediate" or "high". MSI refers to the number of mutations specifically present in satellite regions (special DNA repeat sequences) and it is classified as "low/stable" or "high/unstable".

Dr. Elisabeth Maritschnegg, Product Manager for TMB and MSI analyses at CeGaT, explains why the use of these two analyses have become so widespread in the Oncology setting: "Immunotherapy has revolutionized the treatment of many types of cancer, with an increasing number of immunotherapy-based clinical trials launched in the last few years. Biomarkers are, however, necessary to stratify patients according to their response to treatment, as well as to support the promising development of new immunotherapy combinations. Two biomarkers that have

Services

- Standard Exome, Genome and Transcriptome Sequencing
- Somatic Tumor Panel Sequencing
- Liquid Biopsy Analysis
- Tumor Mutational Burden (TMB) and Microsatellite Instability (MSI) Analysis
- T-cell Receptor Sequencing
- Human Leukocyte Antigen (HLA) Analysis
- Microbiome Analysis

Selected projects - clinical studies

- Neoantigen Vaccination
- Molecular Profiling of Tumor and Tumor Microenvironment
- Treatment Monitoring through Liquid Biopsies

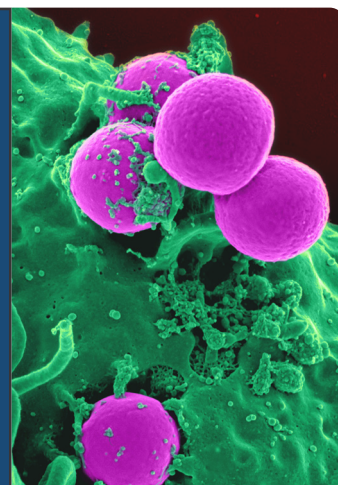


Table I: CeGaT's NGS activities in the field of PM-Oncology

been identified as particularly useful for immunotherapy are TMB and MSI. Patients that are TMB high and MSI high, for example, are shown to have a better response to immune checkpoint inhibitors”.

CeGaT's Methods for TMB and MSI Analysis

CeGaT offers three different [approaches](#) (sequencing panels) for determining the TMB and MSI Score: (1) Their own CeGaT somatic tumor panel, (2) Illumina's TruSight Oncology 500 (TSO500) tumor panel, and (3) Whole-Exome Sequencing (WES) based on Twist Bioscience's chemistry. CeGaT's somatic tumor panel and Illumina's TSO500 interrogate 750 and 523 cancer-related genes, respectively. Approximately 20,000 genes are analyzed through the WES approach.

From Manual to Automated NGS Library Preparation

Due to the high sample throughput for TMB and MSI analysis, CeGaT decided to automate two of the three previously mentioned protocols for NGS library preparation; The protocol for analysis through their in-house somatic tumor panel and the one for analysis through WES. “Automation was a clear choice for us from early on because we wanted to ensure the necessary throughput, as well as the traceability of the samples and the reproducibility of the results” says Dr. Isabell Haake, Team Leader Automation.

Currently, CeGaT has state of the art automated systems for TMB and MSI analysis, including Qiagen's QIASymphony™ and ThermoFisher Scientific's KingFisher™ purification systems for nucleic acid extraction, Hamilton's Microlab® STAR™ liquid handling platforms for NGS library preparation and Illumina's MiSeq® and NovaSeq™ 6000 sequencers for nucleic acid sequencing (Figure 1).

Automated NGS Library Preparation

As a result of their focus on innovation, CeGaT is continuously expanding its product portfolio in order to account for the latest developments in the field of genetic diagnostics. Therefore, the automation solution that CeGaT looked for, required significant flexibility to allow for this continuous development. “The most important decision criteria was the possibility to program relatively easy and fast by ourselves. The Hamilton liquid handling platforms gave us all the flexibility we needed to develop and automate our NGS library prep workflows”, says Dr. Isabell Haake, the leader of the automation team. In addition, CeGaT was attracted by the flexibility of the Hamilton platform's decks, the variety of consumables offered and the technical features of the channels and modules, as well as the positive feedback from other users (Table II).

- The intuitiveness and ease of use of the software
- The possibility to program on our own
- The flexibility of the deck design
- Good customer support

Table II: CeGaT's favorite features in Hamilton's systems

Description of the System and the Workflows

CeGaT uses two customized Microlab® STAR™ platforms for NGS library preparation. Each Microlab® STAR™ is equipped with eight 1000 µL CO-RE® channels, a CO-RE® 96 Probe Head, a CO-RE® Gripper (for plate transfer), a Hamilton Heater Shaker™ (HHS) and an Alpaqua magnet (for clean-up steps), an INHECO Cold Plate Air Cooled (CPAC, for temperature control of the samples), and a Hamilton On-Deck Thermal Cycler (ODTC, for cycling steps) (Figure 2).

Nucleic Acid Extraction →

NGS Library Prep →

Sequencing



Qiagen's
QIASymphony™



Hamilton's
Microlab® STAR™



Illumina's
NovaSeq™ 6000

Figure 1: Fully automated system for TMB/MSI analysis

1000 µL CO-RE® Channels

CO-RE® 96 Probe Head

CO-RE® Gripper

MICROLAB® STAR™ PLATFORM

TOOLS AND MODULES

Autoload

Cold Plate Air Cooled (INHECO)

HAMILTON Heater Shaker™

Magnet (Alpaqua)

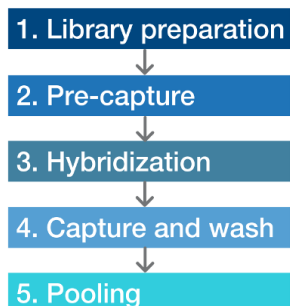
On-Deck Thermal Cycler

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Figure 2: Tools and modules used on the Hamilton Microlab® STAR™ during the NGS sample preparation workflows automated by CeGaT

The workflows for NGS library preparation of the two automated methods used for TMB/MSI analysis (in-house somatic tumor panel and the WES) is similar and includes five main general steps (Figure 3):

Figure 3: Main steps of CeGaT's workflows for NGS library preparation



All steps are performed on-deck except step 3, hybridization, which CeGaT chooses to do off-deck due to its long duration. This allows them to free-up the deck for the analysis of more samples. Slight modifications are made to the deck layout of the Microlab® STAR™ to enable running the complete workflow on the same platform. The preparation for the quality control of the library is also performed on the Microlab® STAR™.

Benefits

The flexibility of the Microlab® STAR™ platform and software, allowed CeGaT to automate two of the methods for NGS sample preparation that can be used for TMB/MSI analysis. One of these methods, the Twist Human Core (Twist Bioscience), was developed and validated within only four months. A commercial version of this automated method is currently in development in collaboration with Hamilton.

Thanks to its high level of automation and state of the art technology, CeGaT is currently able to process 1,000 exome samples per week or 50,000 exome samples per year. It must be highlighted, however, that these protocols are not only used for TMB/MSI analysis, but also for other types of analyses. Moreover, the automation of the workflows for NGS library preparation significantly increases the efficiency and reproducibility of the assay by (i) Allowing the processing of more samples per run, (ii) Reducing the hands-on time and (iii) Reducing the probability for errors, thanks to the full traceability of the samples (Table III).

“Thanks to our robotic workstations, we were able to automate NGS workflows despite rapid method development and changing protocols. For the future, we want to expand our portfolio and automate as many methods as possible. Our goal is to ensure the highest quality standards for sample analysis, while remaining adaptable to the rapidly evolving NGS methods”, says Nicole Weiblen, Team Leader Customer Care. CeGaT is currently working on the automation of new NGS-based assays for oncology testing, as well as testing in

other disease areas using Hamilton’s platforms.

	Manual Approach	Automated Approach
Number of samples per run	32 samples	96 samples
Total hands-on time per run	6 hours	3.5 hours
<i>Library Preparation</i>	2.5 hours	1.5 hours
<i>Hybridization</i>	1.5 hours	1 hour
<i>Capture/Wash</i>	2 hours	1 hour

Table III: Comparison between manual and automated NGS library preparation for TMB/MSI analysis



Disclaimer: throughout this case study, protected product names may be used without being specifically marked as such.

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