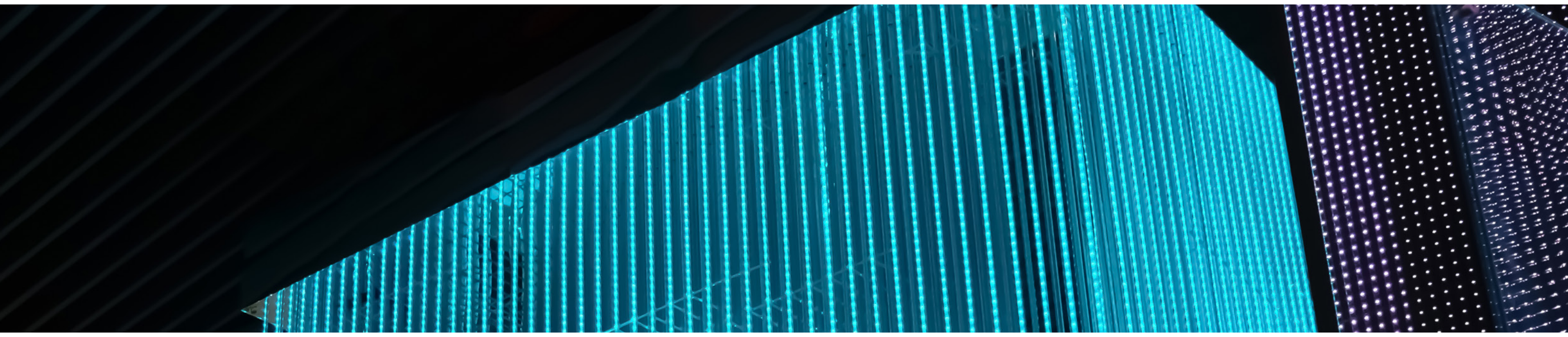
TWIST EF Kit 2.0 Fully Automated on the DISPENDIX G.STATION NGS Workstation

Dr. Felicia Truckenmueller, Head of Applications
DISPENDIX GmbH, Stuttgart, Germany



Efficient fully automated NGS Library Preparation

Next Generation Sequencing (NGS) enables researchers to elucidate the genetic composition of individuals, to characterize gene expression, and to look for rare variants that are associated with disease NGS is an invaluable tool for researchers across a wide range of fields, including oncology research, molecular diagnostics, and even ancestry.

The increasing use of NGS as a routine approach genetic analysis drives the need for robust, high-quality and reproducible library construction that can be executed by any laboratory technician. The G.STATION has been designed and built to construct NGS libraries in a fully automated manner. It simply requires users to load reagents and samples onto the workstation, to press start and then to walk away to come back up to 96 sequencing ready NGS libraries.

A fully automated solution for NGS Library Prep enables researchers to allocate their time to more intellectual contributions around the NGS workflow, such as data analysis or the planning of downstream experiments rather than manually pipetting and moving microplates between instruments.
Key benefits of the G.STATION include:

- Ultra-low dead volume non-contact reagent dispensing to save tips and prevent carryover
- On-deck thermal cycler and temperature/shaking modules for automated incubations and amplification
- Automated, tip-free, bead-based clean-ups for high yield and high purity DNA purification
- Fully traceable process from sample to library
- A library of standardized and tested protocols available in the DISPENDIX cloud platform.

The G.STATION contains all the tools necessary to accomplish successful library prep, by combining the L.DROP Liquid handler with on deck thermal cycling, shaking and temperature control with the C.WASH, a tip-free solution for the bead-based cleanup, and the I.DOT, a non-contact enzyme dispenser, all of which are accessible and connected by the L.MOVE, a collaborative robotic arm.

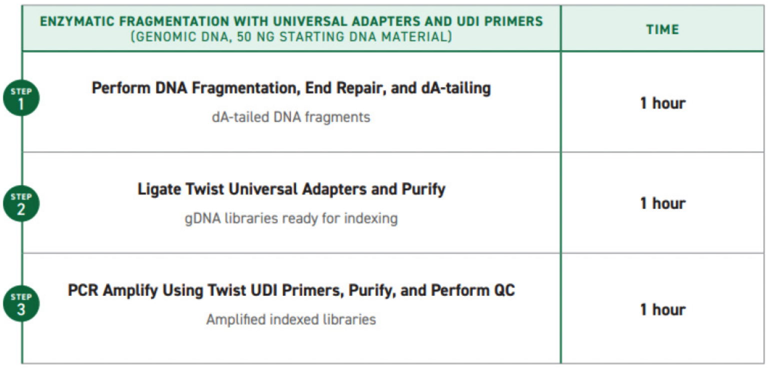
The Twist Library Preparation Enzymatic Fragmentation (EF) 2.0 Kit contains the reagents for the library preparation of genomic DNA (gDNA). It uses enzymatic gDNA fragmentation and the Twist Unique Adapter System. The resulting libraries are compatible with Illumina®-sequencing platforms. The yield and size of the libraries generated with the G.STATION are equivalent to manual preparation, while the automated workflow offers a few significant advantages:

- Significant reduction in hands-on-time
- Standardized workflow for reliable results
- Elimination of pipetting errors
- Less reagent waste due to low dead volume
- Knowledgeable support structure
- Web-based software for remote monitoring and workflow traceability.

Materials and method

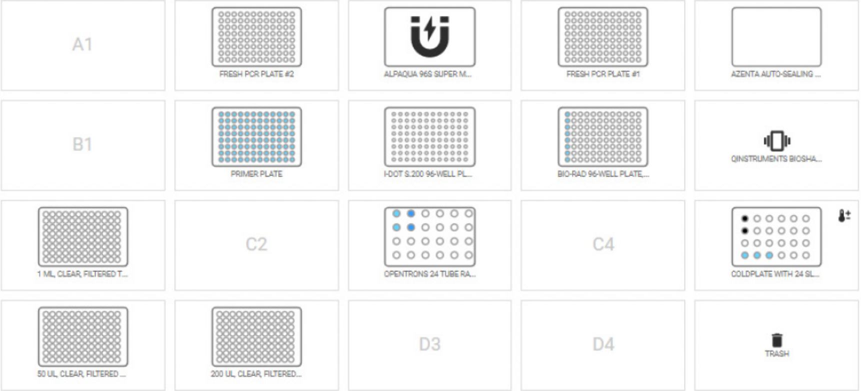
The sequencing libraries were prepared using the G.STATION from 8 samples of 50 ng total mass in 40ul of waterHorizon, Tru-Q NGS DNA Multiplex and the Twist Enzymatic Fragmentation Kit 2.0. The protocol consists of three different steps, first the DNA fragmentation, end repair and dA-tailing. This is followed by the second step of Twist Universal Adapter ligation and purification of fragments. Finally, the library amplification by Twist UDI primers and purification. The protocol takes 3 hours with 10 minutes of hands-on time.

Table 1. *Time requirements for Twist Enzymatic Fragmentation 2.0 Kit.*



	ENZYMATIC FRAGMENTATION WITH UNIVERSAL ADAPTERS AND UDI PRIMERS (GENOMIC DNA, 50 NG STARTING DNA MATERIAL)	TIME
STEP 1	**Perform DNA Fragmentation, End Repair, and dA-tailing** dA-tailed DNA fragments	**1 hour**
STEP 2	**Ligate Twist Universal Adapters and Purify** gDNA libraries ready for indexing	**1 hour**
STEP 3	**PCR Amplify Using Twist UDI Primers, Purify, and Perform QC** Amplified indexed libraries	**1 hour**

The Dispendix Cloud contains the tailored protocol, outlining every step on the graphic interface with real-time progression of the protocol. Further, the deck set up is displayed, for easy deposition of the required reagents.



A1	FRESH PCR PLATE #2	ALPAQUA 96S SUPER M...	FRESH PCR PLATE #1	AZENTA AUTO-SEALING ...
B1	PRIMER PLATE	I-DOT S.200 96-WELL PL...	BIO-RAD 96-WELL PLATE,...	QINSTRUMENTS BIOSHA...
1 ML, CLEAR, FILTERED T...	C2	OPENTRONS 24 TUBE RA...	C4	COLDPLATE WITH 24 SL...
50 UL, CLEAR, FILTERED ...	200 UL, CLEAR, FILTERED...	D3	D4	TRASH

Figure 1. *Deck Layout for 8-sample processing to create NGS libraries with the Twist EF Kit 2.0.*

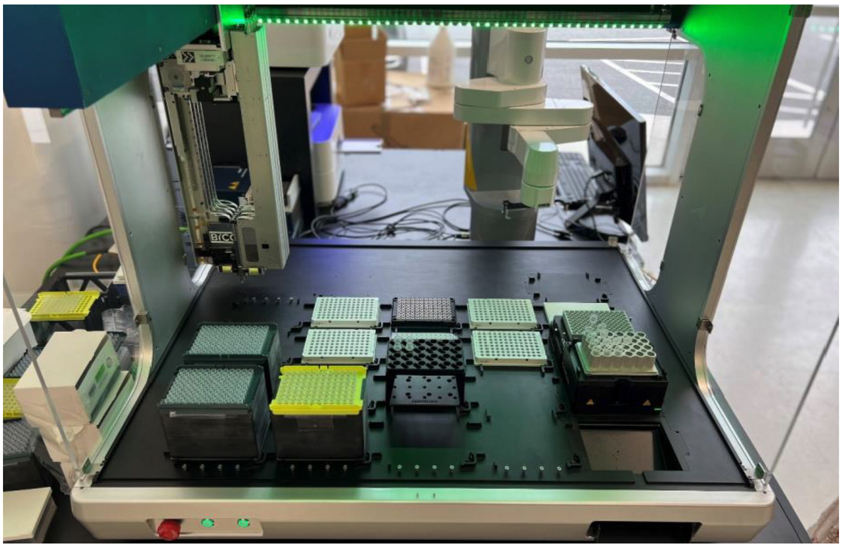


Figure 2. *Deck Set up on the automated Liquid Handler for application start-up, with tip storage, reagent plates, shaker and cooling module.*

Results and discussion

After the last step of library amplification, the resulting libraries were quantified with the Thermo Fisher® Qubit HS assay after an initial 1:5 dilution of the DNA with water. Further, the fragment size was analyzed with an Agilent 2100 Bioanalzyer.

The average DNA concentration of the sequencing libraries is 177,68 ng/µl ± 34,46 ng and proves a successful and sufficient PCR amplification.

The distribution of the peak sizes shows a very homogenous library creation with fragment lengths of 329 bp ± 16 bp on average. This fragment length lies well within the recommended fragment sizing for Illumina-based sequencing. The homogeneity of the libraries is another beneficial aspect for successful sequencing results.

Table 2. *Quantification results of the gDNA libraries prepared by the Twist EF 2.0 kit with Qubit HS DNA assay.*

Sample	Conc. (ng/µl)
1	191.50
2	236.00
3	178.50
4	213.50
5	134.50
6	185.50
7	143.00
8	139.00

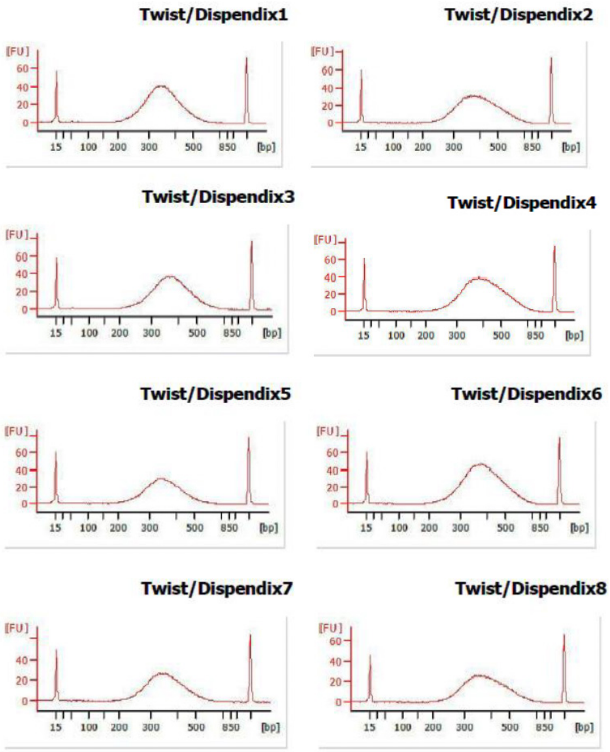


Figure 3. *Peak size distribution of sequencing libraries, analyzed with t he Agilent 2100 Bioanalyzer.*

Conclusion

The G.STATION NGS Workstation provides a full and trustworthy solution for NGS library construction, as demonstrated by the high yield and highly uniform NGS libraries generated through full workflow automation of the TWIST Enzymatic Fragmentation 2.0 Kit.

The G.STATION fully automates the library preparation process from sample to library with zero manual intervention required. The DISPENDIX Cloud Platform provides standardized and tested protocols that can be run by any lab technician regardless of automation experience.

With 10 minutes of hands-on time loading a sample plate and reagents onto the deck of the G.STATION NGS workstation, any lab can quickly produce high-quality NGS libraries within days of installation.

Due to the use of a instruments designed specifically for addressing NGS workflow steps, a reagent dispenser and a bead-based clean-up instrument, the G.STATION utilizes a fraction of the tips required by traditional automated platforms, and reliably generates high-quality libraries with an unparalleled speed and reproducibility.

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