



Twist Library Preparation on Echo 525 Acoustic Liquid Handler and Biomek i7 Automated Workstation

Introduction

This technical note demonstrates the miniaturized Twist Library Preparation EF 2.0 with Enzymatic Fragmentation and Twist Universal Adapter System method on the Echo 525 Acoustic liquid handler and Biomek i7 Hybrid automated liquid handler. The library protocol as described is designed to be followed by the Twist Target Enrichment on the same Biomek i7 Hybrid automated liquid handler.

This Twist Library Preparation Kit provides the reagents needed to prepare genomic DNA (gDNA) libraries using enzymatic gDNA fragmentation and the Twist Universal Adapter System. The Twist Universal Adapter system consists of Twist Universal Adapters and Twist Unique Dual Indexed (UDI). Primers are available in 96- and 384-well formats with up to 3072 unique pairs. This protocol is designed to accommodate greater than 384 in order to provide significant savings of time. The detailed steps below provide demonstration for generating the amplified, indexed libraries needed for downstream target enrichment and sequencing on Illumina next-generation sequencing (NGS) systems. This library preparation protocol is optimized for use with Twist Target Enrichment Kits and should only be performed with reagents specified or their equivalents.

In comparison to the manual pipetting, automating the Twist Bioscience Library Preparation kits on Biomek platforms provides:

- Robust and reproducible library construction
- Reduced hands-on time and increased throughput
- Reduction in potential pipetting errors
- Standardized workflow for improved results
- Quick implementation with ready-to-implement methods
- Library normalization
- Reduced volume quantification
- Knowledgeable support

Spotlight

Biomek i7 Hybrid Automated workstation

- 300 μ l or 1200 μ l Multichannel head
- Span-8 pod with fixed and disposable tips
- Enhanced Select Tip pipetting to transfer custom array of samples
- Independent 360° rotating gripper with offset fingers
- High deck capacity with 45 positions
- Orbital Shakers, Peltiers, Span-8 and 96-channel tip washing for controlling sample processing
- Spacious, open platform design to integrate on-deck and off-deck elements (e.g. thermo cyclers)

Echo 525 Acoustic Liquid handler

- Dynamic Fluid Analysis: Real-time adjustment to fluid properties enables users to rapidly normalize and pool libraries using non-contact acoustic transfer. No user calibration is required
- Nanoliter-scale Transfers: Enables reduction of NGS library preparation costs by miniaturizing to 1/5th
- Echo Software: Intuitive interface to quickly create transfer protocols with built-in simulators

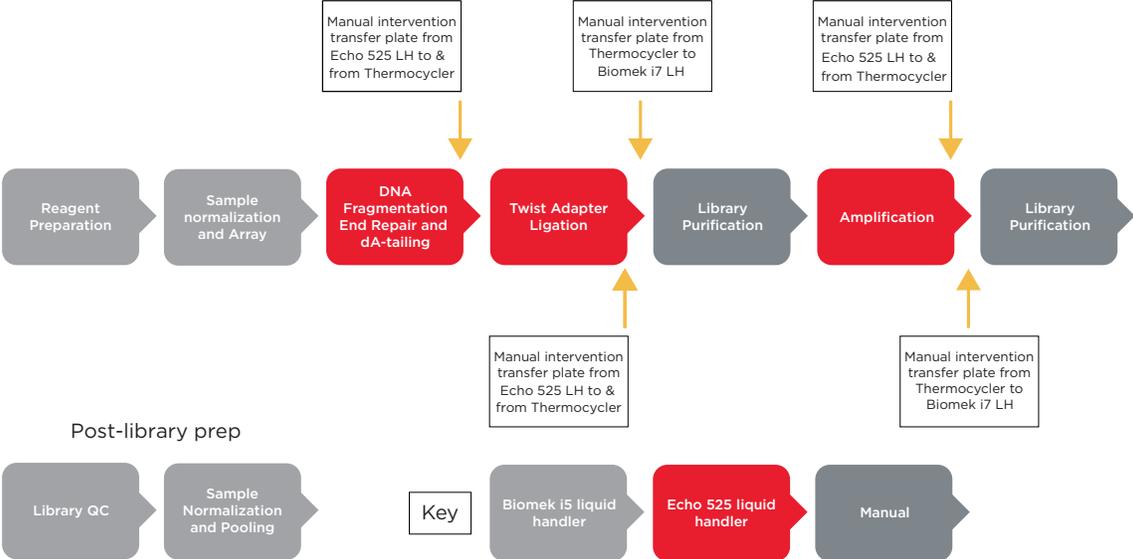


Figure 1. Twist Library Preparation workflow with standalone liquid handling instruments.

Automated method

In this automated workflow there are a total of three individual method components written in Echo Plate Reformat software and a single Biomek 5 software method responsible for the bead cleanup portion of the library protocol.

Batch Size	Hands On-Time (hr:min)	Echo Processing Time (hr:min)	Biomek Processing Time (hr:min)	Total Time (hr:min)
384	0:15	0:30	1:00	3:00

Figure 2. Estimated run times for the Twist Library Preparation EF 2.0 with Enzymatic Fragmentation and Twist Universal Adapter Kits on the Echo 525 Acoustic Liquid Handler and Biomek i7 Dual Hybrid Automated workstation. Timing estimates based on demonstration data.

Biomek software provides several user-friendly features such as:

1. Method Options Selector (MOS)

MOS enables selection of plate processing and sample number options to maximize flexibility, adaptability, and the ease of method execution.



Figure 3. Biomek Method Options Selector enables us to select the desired workflow, sample number, and a variety of workflow customization options.

2. Guided Labware Setup (GLS)

GLS is generated based on options selected in the MOS, and provides the user specific graphical setup instructions with reagent volume calculation and step-by-step instructions to prepare reagents.

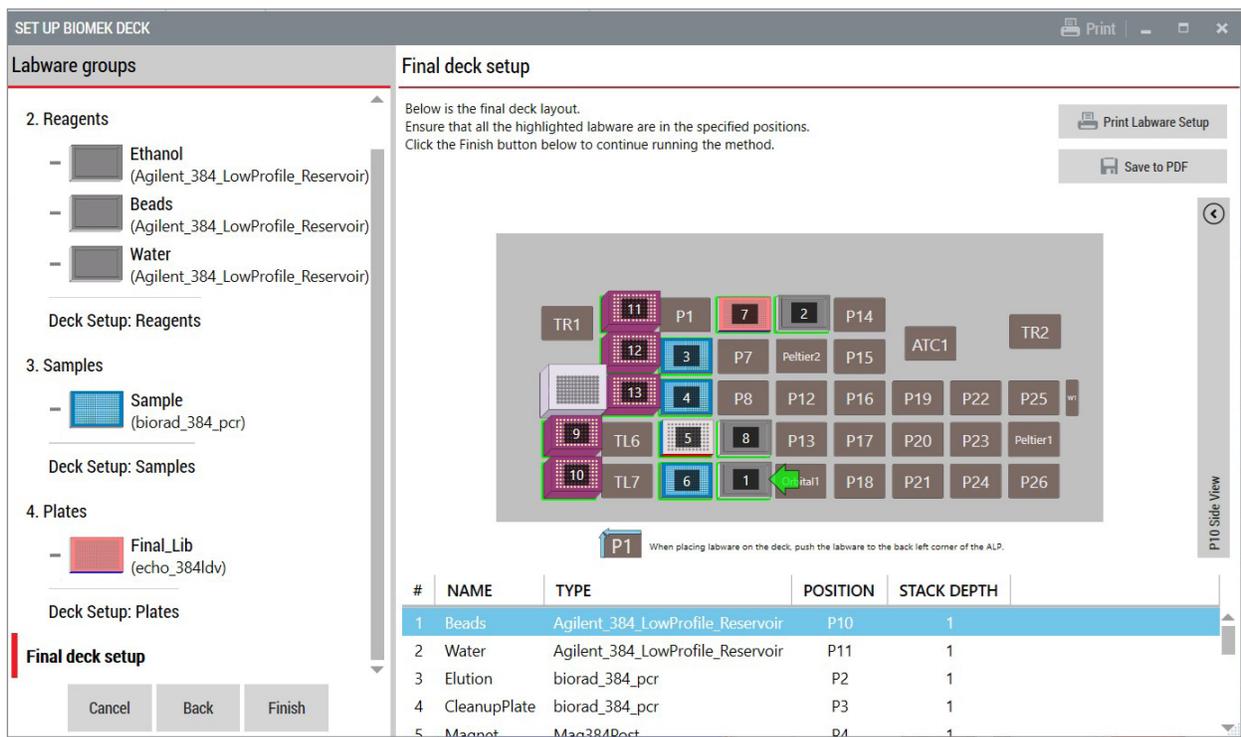


Figure 4. Guided Labware Setup indicates reagent volumes and guides the user for correct deck setup.

Experimental Design

Corielle DNA was manually diluted to 6.25 ng/ μ l and aliquoted into 192 wells of a 384-well plate in a checkerboard pattern, the remaining 192 wells were aliquoted with 8 μ l of nuclease-free water. Fragmentation master mix at a volume of 2 μ l dispensed through the Echo 525 liquid handler from A1 of a Echo 6Res to all 384 wells of the sample plate using the GPSB calibration. The plate loaded into a plate centrifuge at 1500 rpm for 1 min, post-spin the plate was sealed and moved to the 384-well Applied Biosystems ATC for fragmentation and A-tailing reaction. Fragmentation time was set to 5 minutes at 37 °C for the target of 400-600 bp fragment size range. Post-fragmentation the plate was unsealed and moved back into the Echo 525 LH where the ligation master mix was added from A2 at a volume of 4 μ l to all 384-well and the Twist Universal adapter was added via A3 at a volume of 1 μ l to all 384-wells. The plate loaded into a plate centrifuge at 1500 rpm for 1 min, post-spin the plate was sealed and moved to the 384-well Applied Biosystems ATC for 30 minutes at 16 °C. Post-ligation reaction the was unsealed and moved to the Biomek i7 Dual Hybrid for a magnetic bead cleanup at 0.8X ratio. The fragments are eluted and moved into the Echo 525 LH for fragment amplification through dispensing 5 μ l per well of 384-well UDI indexes from an Echo LDV plate, 7.5 μ l of Equinox pcr master mix was transferred into the plate. The plate loaded into a plate centrifuge at 1500 rpm for 1 min, post-spin the plate was sealed and moved to the 384-well Applied Biosystems ATC for 8 cycles of PCR. Post-PCR the plate was unsealed and moved to the Biomek i7 dual hybrid workstation for a magnetic bead cleanup at 0.6X ratio. Post-cleanup each library was quantified using a 384-well quantification method.

Experimental Results

The sequencing-ready libraries produced in the miniaturized workflow when run through the yielded average size range of 400-600 base pairs with distribution ranging from as low as 300 to 700 base pairs with concentrations from 28 ng/ μ l to 91 ng/ μ l were yield in 191 of 192 wells with sample. One well failed to yield library with a measurable concentration of 0.32 ng/ μ l as visible in Figure 7.

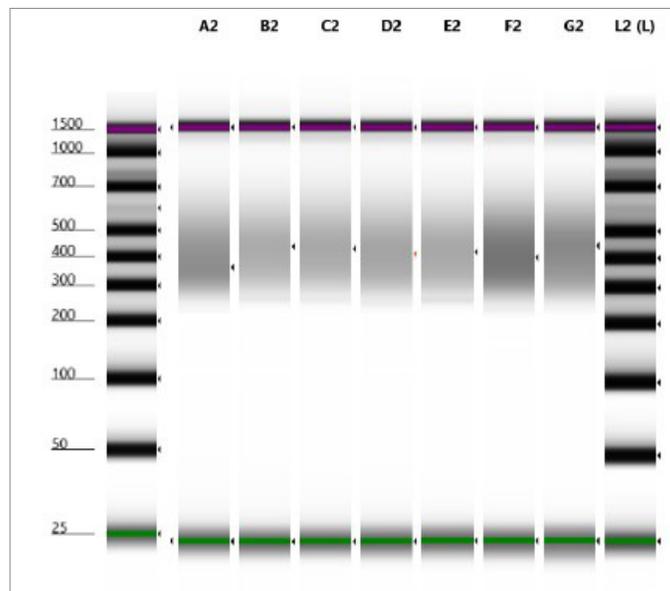


Figure 5. Digital gel image of a subset of samples from Quadrant A1 of the 384-well plate run as described in the experiment above. Each sample was diluted 1 μ l library with 4 μ l diH₂O prior to loading on the Agilent High Sensitivity D1000 ScreenTape System.

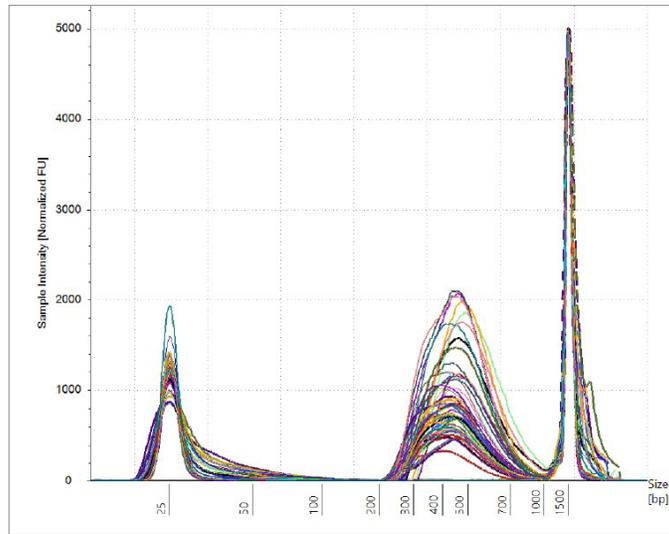


Figure 6. Peaks output overlay of 96 samples from Quadrant A1 ran on the Agilent High Sensitivity D1000 ScreenTape System.

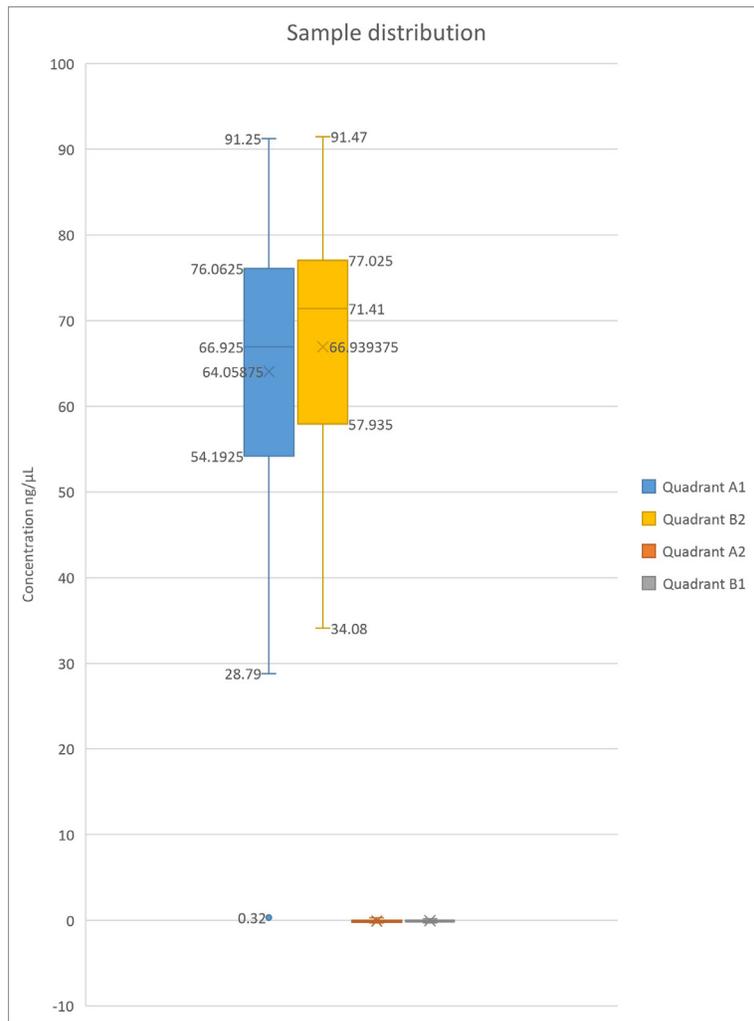


Figure 7. Box plot of the concentration values post-library preparation of the checkerboard plate. Quadrant A1 and B2 contained sample and generated library as expected. Single outlier well that did not yield data found in quadrant A1. Quadrants A2 and B1 were no sample control wells.

Summary

Quantifiable libraries in the range of 28 ng/μl to 91 ng/μl were yield in 191 of 192 wells with sample. The average fragment length distribution across those wells was between 400-600 base pairs. Miniaturization of the library preparation workflow on the Echo 525 Automated Liquid Handler when combined with the Biomek i7 Dual Hybrid Automated Workstation allows for the user to create robust and reproducible libraries for the Twist Hybridization workflow.

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Echo Acoustic Liquid handlers and Biomek Automated Workstations are not intended or validated for use in the diagnosis of disease or other conditions.

Biomek Method Launcher software package has to be purchased separately.

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