Automating NGS Library Preparation Using Twist Bioscience Enzymatic Fragmentation Kit 2.0 and Universal Adapters System on Opentrons Flex™

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ABSTRACT

Preparing next generation sequencing libraries involves a multi-step process to fragment DNA, repair their ends, add adapters, and amplify the resulting DNA. This process, which involves multiple clean up steps in between, requires the user to frequently come back to the experiment between incubations. Automating this entire process in one protocol allows the user to step away from the bench to do other tasks. Here, we describe an automated protocol for the Opentrons Flex to prepare sequencing-ready libraries using the Twist Library Preparation Enzymatic Fragmentation (EF) Kit 2.0 and Twist Universal Adapter System.

Key Features

- Automating enzymatic fragmentation and library preparation on the Opentron Flex produces consistent DNA fragments typically averaging ~300 base pairs in length.
- Combining multiple NGS library preparation protocols into one automated workflow allows the user to set up the protocol and then step away for the entire duration of the protocol.

INTRODUCTION

Fragmentation is an important step in next-generation sequencing library preparation. Because NGS platforms do not use whole pieces of DNA, DNA molecules need to be broken into fragments of 145-425 base pairs before they can be used to build libraries. DNA fragmentation can be done either (a) mechanically using sonication or acoustic shearing, or (b) enzymatically using restriction enzymes, transposases, or other enzymes.

Enzymatic fragmentation may be chosen over mechanical methods because it's less damaging to the DNA, scalable, and doesn't require specialized instruments. Because of this, enzymatic fragmentation also lends itself to automation and can be easily coupled with adapter ligation and library amplification in an automated protocol. By combining different steps of the library prep protocol together, automation conserves scientists' time by eliminating the need to manually intervene after each incubation step to set up the next part of the protocol.

In this app note, we describe how to automate enzymatic fragmentation, adapter ligation, and PCR amplification using the Twist Library Preparation EF Kit 2.0 and Twist Universal Adapter System on the Opentrons Flex robot.

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METHODS

Samples

50 ng of Promega human male genomic DNA (cat. no. G1471) was used as a starting input for enzymatic fragmentation.

Enzymatic fragmentation and library preparation

We used the Twist Library Preparation Enzymatic Fragmentation Kit 2.0, 96 samples (cat. no. 104207) to fragment DNA and the Twist Universal Adapter System TruSeq Compatible, 96 Samples Plate A (cat. no. 101308) to add adapters to the fragmented DNA followed by amplifying on the unique dual indices using UDI primers (Figure 1). The protocol was automated on the Opentrons Flex NGS Workstation (cat. no. 991-00179) using the Twist Bioscience Library Preparation EF 2.0 with Enzymatic Fragmentation and Twist Universal Adapter System protocol (DOC-001239 REV 6.0; library.opentrons.com/p/Twist-EF-2_0) with the default protocol parameters listed in Table 1.

Figure 1. Fully automating the Twist BioScience Library Preparation EF 2.0 workflow on the Opentrons Flex reduces hands-on time by by 67%. Timings are approximate for 8-sample throughput.

Table 1. Protocol parameters used for automation.

Parameter	Input type	Default*	Minimum	Maximum	Description
Dry Run	Bool	False			Dry runs will skip incubations on the thermocycler
On Deck thermocycler	Bool	True			Will you be using an Opentrons thermocycler?
Samples	Integer	8	1	24	How many samples will be processed?
PCR cycles	Integer	7	3	12	Number of Amplification cycles
Fragmentation Temperature	Integer	37	30	37	Fragmentation temperature in celsius
Fragmentation time	Integer	20	5	40	Desired fragmentation time in minutes
Adapter Volume	Integer	5	1	5	Volume of Adapter added in ul
Starting column on primer plate	Integer	1	1	12	Choose the column on primer plate where you would like to start adding primers from (useful for partially used plates)

*Default conditions are based on the standard Twist protocol; please consult Twist Technical Support if modifications are required.

Analysis of DNA library:

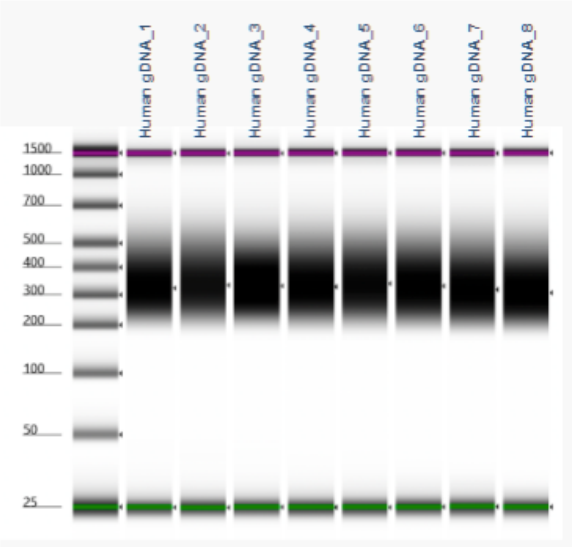
Once prepared, DNA libraries were analyzed on the Agilent 4150 TapeStation using the High Sensitivity D1000 Reagents (cat. no. 5067-5585) and High Sensitivity D1000 ScreenTape (cat. no. 5067-5584). DNA was quantified using the Qubit™ 1X dsDNA High Sensitivity Assay Kit (cat. no. Q33231) and the Qubit™ 4 Fluorometer.

RESULTS

Samples

Using the Twist Bioscience Library Preparation EF 2.0 with Enzymatic Fragmentation and Twist Universal Adapter System protocol (DOC-001239 REV 6.0; library.opentrons.com/p/Twist-EF-2_0), we completed DNA fragmentation and library preparation for eight samples in 2 hours and 40 minutes on the instrument using the default parameters. Before automation, hands-on time of about 30 minutes was required to set up the deck and prepare reagents and sample plates. When we expanded to 16 samples and 24 samples, automated library preparation time on the instrument increased to 3 hours and 3 hours and 30 minutes, respectively. For different scenarios, the actual run time would be different depending on the fragmentation time and number of PCR cycles used. If needed, users can shift protocol parameters between the minimums and maximums as defined in Table 1.

Subsequently, we visualized and quantified the yield and fragment size of the 8-sample run. The gel view shows fragments primarily between 300 and 500 base pairs (Figure 2). The final concentration of each library averaged 71.6 ng/µl with an average fragment size of 328.1 bp.



	Yield (ng/µL)	Size (bp)
Average	71.6	328.1
CV	13.3	3.36

Figure 2. High-yielding and uniform libraries generated from human gDNA. The data shown is from 8 samples of Promega human male genomic DNA using 50 ng input.

DISCUSSION

Automating DNA fragmentation and adapter ligation on the Opentrons Flex using the Library Preparation Enzymatic Fragmentation Kit 2.0 and the Universal Adapter System from Twist Bioscience saves scientists a substantial amount of hands-on time. With as little as 30 minutes of hands-on time to set up the deck and prepare reagents and sample plates, the rest of the process can be completed without manual intervention. Runs consisting of 8 to 24 samples can be automated in about 3 to 3.5 hours after set up.

By automating DNA fragmentation and library preparation in one protocol, we enable users to set up reagents and step away for the remainder of the entire protocol. This contrasts with manual methods where users must come back to the prep at several time points (e.g., to set up fragmentation, to set up ligation, to set up PCR amplification). In summary, Opentrons Flex paired with Twist EF 2.0 Library preparation workflow for automating DNA fragmentation and library preparation produces consistent, high-yield libraries with little human intervention throughout the protocol.

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