

Easy Illumina® Nextera® DNA FLEX Library Preparation using the epMotion® 5075t automated liquid handler

Executive Summary

Library preparation steps, including DNA extraction, quantification, fragmentation, normalization, purification and quality control pose major labor-intensive bottle necks for the generation of sequencing data.

With the release of the Nextera DNA Flex Library Preparation kit, Illumina introduced a new chemistry which streamlines these time-consuming steps for a faster, highly flexible workflow. To even further streamline the library preparation, Eppendorf provides an Illumina qualified epMotion method as ready-to-run automated solution with minimal hands-on time.



Introduction

Whole genome sequencing offers the most comprehensive tool for genomic studies. Still a demand exists for flexible, yet more streamlined workflows. The Nextera DNA Flex Library Prep kit chemistry offers such a workflow for whole-genome sequencing applications. Here we illustrate the simplicity and robustness of automating this workflow on the epMotion.

Key highlights

- > Full flexibility to accommodate variations in sample type, DNA input amount, and number of samples from 1 to 96
 - > Library preparation is compatible with the 24- and 96- sample-versions of the kit, allowing several runs with kit supplied reagents
 - > Automated handling of potentially hazardous body fluids, such as saliva
 - > Maximized library yield and quality by using high-quality Eppendorf LoBind® consumables and equipment such as the Mastercycler® X50.
- > Illumina qualified procedure to generate high quality, sequencing ready libraries and reliable sequencing results
 - > Easy-to-setup platform requiring minimal user training
 - > Low to intermediate sample throughputs (up to 96 samples)
 - > Automation maximizes walk-away time, frees up time and reduces the likelihood of human error and variability

Solutions & Benefits

Workflow: Library preparation in just 4 steps

On the epMotion, Nextera DNA Flex users have full flexibility over which processes they wish to automate and from which steps they may want to proceed. The entire workflow is divided into only 4 methods for processing up

to 96 samples in parallel as shown in Figure 1. The epMotion can accommodate different sample inputs and provides full flexibility as to which kit version (i.e. 24-plex or 96-plex) the user chooses.

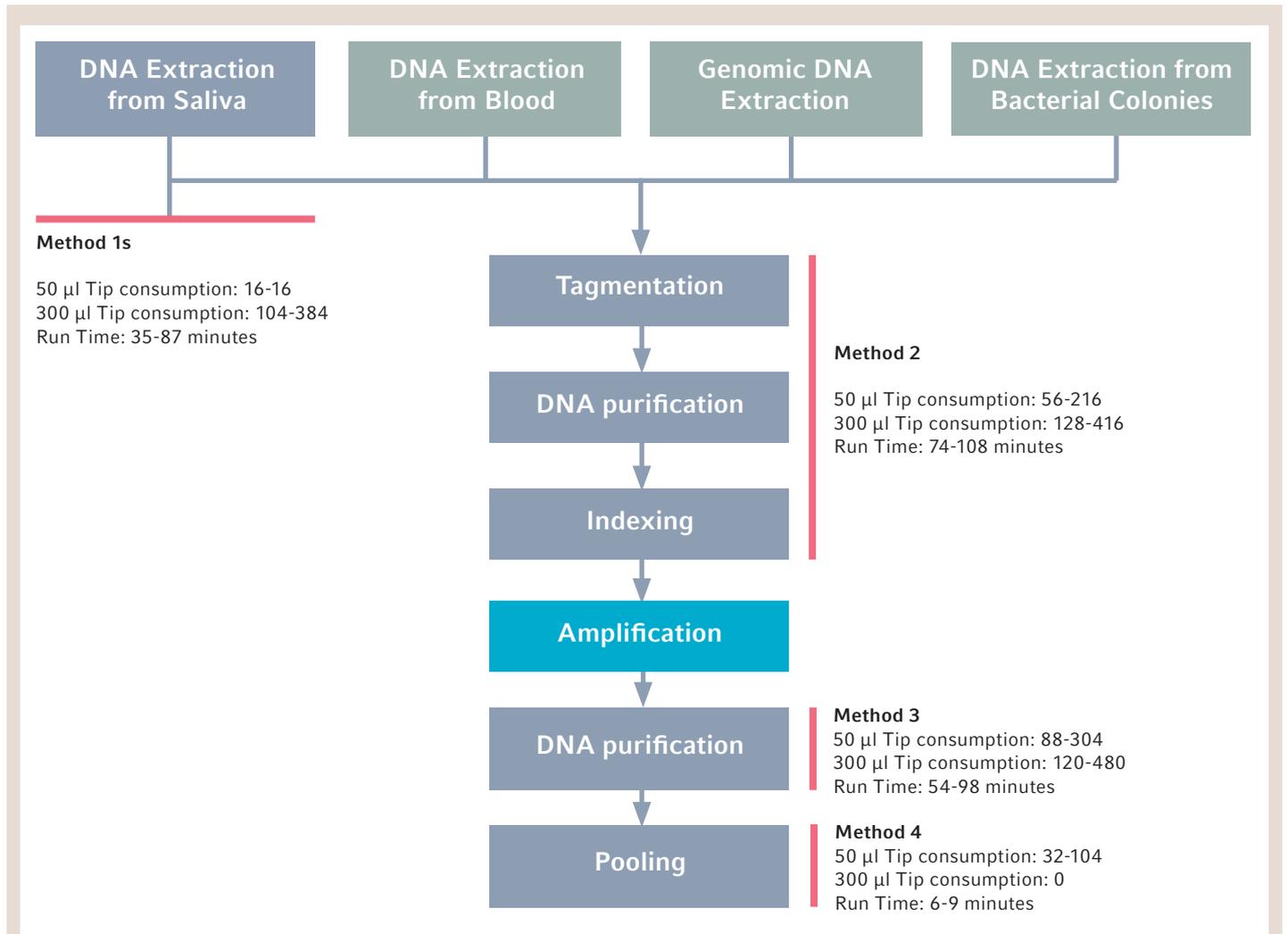


Figure 1: Workflow for the Nextera DNA Flex Library Preparation protocol on the epMotion showing single steps. Red boxes show which steps are part of which method file. Steps highlighted in green are not part of the qualified method but can be performed using the epMotion. The running times on the epMotion and tip consumption are shown for handling 24 and 96 samples for each method. The complete process allows construction of 96 sequencing-ready libraries in less than a day with hands-on time of less than one hour.

Generate high-quality libraries with the automated workflow on the epMotion

When investing into a method, it is important to have as little variation as possible and to reduce the risk of wasting costly reagents and labor time. The epMotion allows the generation of ready to sequence libraries with minimal setup time. To demonstrate the reliability of the method 8 libraries were prepared from 200 to 300 ng DNA from Coriell Institute female reference cell line NA12878 and sequenced in two independent experiments starting at Method 2. The average fragment size was closely clustering around ~600 bp as

recommended by Illumina (Figure 2). Library yields were highly reproducible and comparable to manual data (Table 1). Furthermore, the median sequencing insert size clustered tightly around the 350 bp optimum and is comparable to manual library preparations (Table 1). This translated to good sequencing performance data with high read diversity and even coverage across the genome and autosomes and a low coefficient of variance (CV).

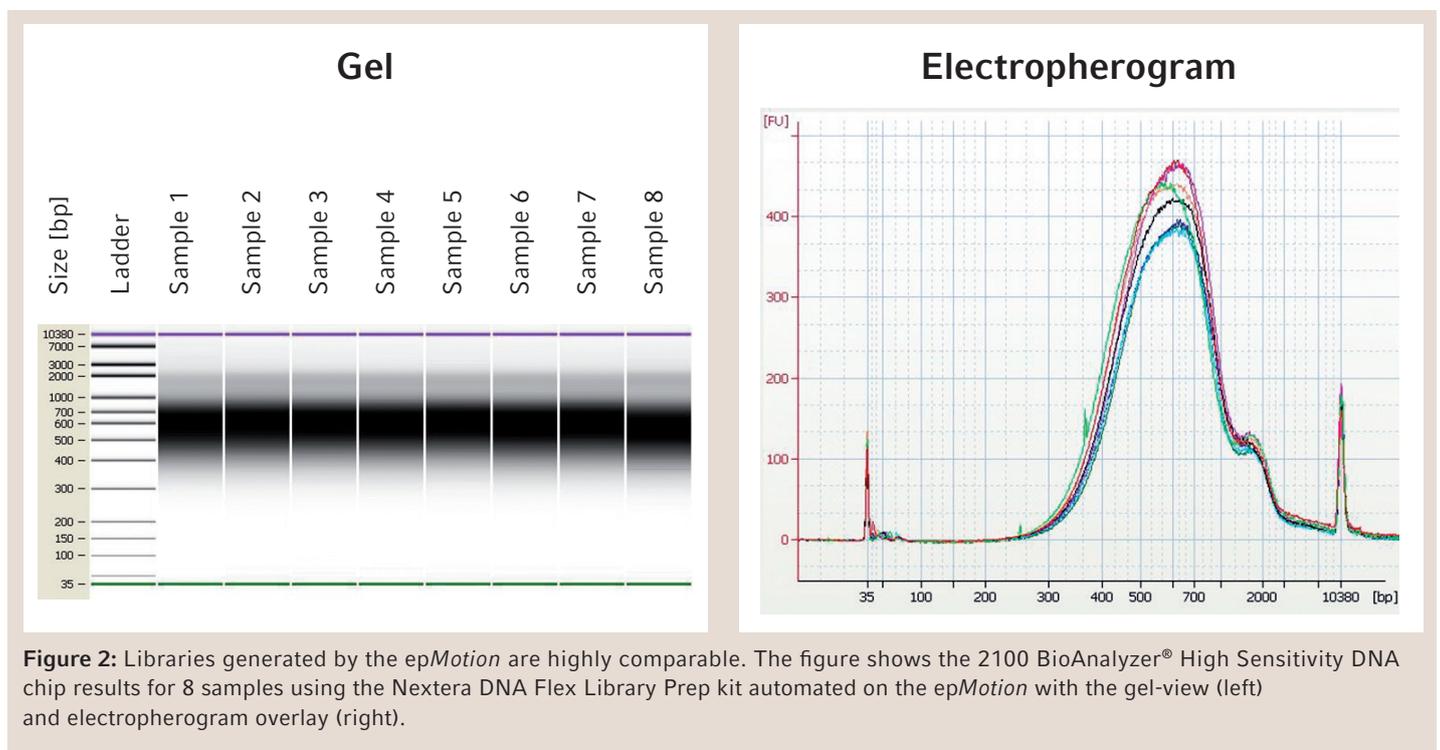


Figure 2: Libraries generated by the epMotion are highly comparable. The figure shows the 2100 BioAnalyzer® High Sensitivity DNA chip results for 8 samples using the Nextera DNA Flex Library Prep kit automated on the epMotion with the gel-view (left) and electropherogram overlay (right).

Table 1: Comparison of results obtained with manual and automated library preparation. Data presented is an average of two 8-plex runs on a HiSeqX® system, with sequencing reads trimmed to a 30X depth (380 million reads, 2 x 151 bp). Data analysis performed using the BaseSpace Sequence Hub Whole Genome Sequencing v5.0 App.

	Manual Preparation	Automated epMotion® 5075t
Yield (Qubit, ng/μL)	13.2	13.6
Yield CV (%)	5.1	3.5
Index CV (%)	10.8	11
Median insert size range (bp)	352	339
Mean diversity	> 2.3e ⁹	>3.0e ⁹
Autosome mean coverage (Fold)	30 - 32x	30 - 32x
Autosome Callability (%)	95.0	95.5

To further demonstrate the efficiency of the upstream saliva lysis protocol, Saliva was collected from a healthy volunteer. This was used as input for an exemplary library preparation with 8 replicates on the automated workflow. The results show good coverage across the human genome, with tight clustering of indexes (table 2). The minor differences in library yield, diversity and percent of aligned reads may result from variability between the input samples, such as bacterial content.

Table 2: Libraries were prepared from 30 µl of the heat treated human saliva material, collected in Oragene® collection tubes (DNA Genotek). Equal volumes (5 µl per sample) of 8 Nextera DNA Flex libraries were pooled together without additional normalization. After paired end 2x151 bp HiSeqX sequencing run, sequencing reads were trimmed to 380 million reads. Data analysis was performed using the BaseSpace Sequence Hub Whole Genome Sequencing v5.0 App.

Metrics	Automated epMotion library preparation 5075t/tc
Yield (ng/µl)	11.3
Yield CV (%)	13.1
Index CV (%)	14.5
Median insert size range (bp)	321
Median diversity	2e9
Autosomal mean coverage (fold)	28
Autosomal callability (%)	94.9

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Eppendorf is a leading life science company that develops and sells instruments, consumables, and services for liquid-, sample-, and cell handling in laboratories worldwide. Its product range includes pipettes and automated pipetting systems, dispensers, centrifuges, mixers, spectrometers, and DNA amplification equipment as well as ultra-low temperature freezers, fermentors, bioreactors, CO₂ incubators, shakers, and cell manipulation systems. Associated consumables like pipette tips, test tubes, microtiter plates, and disposable bioreactors complement the instruments for highest quality workflow solutions.

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