Next-generation sequencing library preparation on firefly – a compact, novel liquid handler

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Overview

We demonstrate that firefly can successfully automate the NEBNext Ultra II FS DNA library preparation workflow to generate high quality sequencing libraries.

Introduction

Next-generation sequencing (NGS) library preparation is a multi-step process, involving numerous pipetting and incubation steps, making it a desirable workflow to automate. Here we demonstrate that firefly, a compact, novel liquid handler, can be used to generate high quality libraries for next generation sequencing.

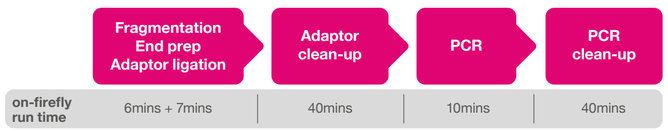
firefly is an extremely compact automated liquid handling platform (width 66cm x depth 56cm x height 78cm), making it easy to place in any laboratory.

firefly consists of:

- two moving decks – with a total of 16 deck positions.
- two liquid handling heads: an air-displacement pipetting head and a non-contact positive-displacement dispensing head.
- The air-displacement pipetting head is comprised of 384 pipetting channels and can aspirate and dispense from both 96 and 384 well plates, depending on the format of the tip array presented to the head.
- The positive displacement head can dispense up to 6 different reagents to 96 and 384 well plates on the deck.
- a gripper to move labware.
- temperature controlled reservoirs for the dispensing head reagents.
- separate heater and shaker modules.

Method

The NEBNext Ultra II FS DNA workflow was split into 4 sub-protocols which were run in turn on firefly. Between each sub-protocol, dispense head syringes and reagents were replaced, and pipetting head tips were replenished.



Key points of the NEBNext Ultra II FS DNA workflow

- Enzymatic shearing of gDNA
- Fragmentation and End prep (end repair + dA-tailing) are performed in one reaction
- NEB adaptors were used – these adaptors have a hairpin loop structure that contains a uracil base
  - Uracil is removed by an enzyme mix (USER) to open the loop and enable PCR
- The protocol was streamlined by implementing modifications used in the ARTIC-NEB: SARS-CoV-2 protocol
  - USER/PCR dispenses and incubations were combined

Run setup

- 100ng Human gDNA input for each test sample (Promega)
- Protocol options
  - Fragmentation-End prep incubation: 30min 37°C, 30min 65°C
  - Adaptor clean-up: No size selection. One-sided clean up.
  - PCR: 4 cycles
- Magnet used: Alpaqua Magnum FLX (96 well ring magnet)
- Working plate: 96 well PCR plate (eppendorf twin.tec)
- Samples prepared: 48 samples (6 columns) including “no template” controls (NTCs)

Library analysis

- All samples were diluted 1:3 in 1X TE buffer then run on a fragment analyser 5200 (Agilent) using a High Sensitivity NGS Fragment kit (Agilent).

Consumables

Consumable type	Number required
Pipetting head tip boxes	14
Dispensing head syringes	12
Dispensing head reservoirs	12
Plate: Empty 96 well PCR plate	3
Plate: Empty 96 well waste plate	1

The number of consumables was based on:

- No reuse of pipetting head tips
- No reuse of dispensing head syringes - except for syringes used to dispense 80% ethanol
- gDNA being supplied in a 96 well source plate
- Indexes being supplied in a 96 well plate

The tables below show the steps that were executed on firefly to process samples through the NEBNext Ultra II FS DNA workflow. The time taken to run groups of the steps is indicated. For dispensing head steps, the time to taken to dispense reagent to all wells of a 96 well plate is also indicated. The total time taken to execute the workflow, from end to end was ~4 hours.

Steps

Key: Dispense head | Pipetting head | Process module | Off-deck

Fragmentation | End-prep | Adaptor ligation

Location	Head / Process module	Reagent	Volume (uL)	Destination	Details/Dispense time
On-deck	**Dispense**	FS enzyme+ buffer	**9**	PCR plate 1	1.5min
	Pipetting	DNA input	**26**	PCR plate 1	
	Shaker - mix			PCR plate 1	1min 1500rpm
Off-deck	**Thermocycler** Fragmentation / end prep			PCR plate 1	30min 37°C 30min 65°C
On-deck	**Dispense**	Adaptor	**2.5**	PCR plate 1	1min
	Dispense	Ligation MM + enhancer	**31**	PCR plate 1	2mins
	Shaker - mix			PCR plate 1	1min 1500rpm
Off-deck	**Thermocycler** Adaptor ligation			PCR plate 1	15mins 20°C

6 mins

7 mins

Adaptor clean up

Location	Head / Process module	Reagent	Volume (uL)	Destination	Details/Dispense time	Magnet
On-deck	**Dispense**	Beads (0.8X)	**55**	PCR plate 1	3mins	
	Pipetting: Tip Mix			PCR plate 1	6mins	
	Heater			PCR plate 1	5mins 23°C	
	Pipetting	Supernatant → waste		Waste		On-magnet
	Dispense	80% EtOH	**60**	PCR plate 1	1min + 2min	On-magnet
	Pipetting	EtOH → waste		Waste		On-magnet
	Dispense	80% EtOH	**60**	PCR plate 1	1min + 2min	On-magnet
	Pipetting	EtOH → waste		Waste		On-magnet
	Dispense	Elution buffer	**17**	PCR plate 1		
	Pipetting: Tip mix			PCR plate 1		
	Heater			PCR plate 1	2mins 23°C	
	Pipetting	Transfer	**15**	PCR plate 2		On-magnet

40 mins

PCR

Location	Head / Process module	Reagent	Volume (uL)	Destination	Details/Dispense time
On-deck	**Dispense**	PCR MasterMix + USER	**25**	PCR plate 2	2mins
On-deck	**Mix - Shaker**			PCR plate 2	1min 1500rpm
On-deck	**Pipetting**	Indexes	**10**	PCR plate 2	
On-deck	**Mix - Shaker**			PCR plate 2	1min 1500rpm
Off-deck	**Thermocycler PCR**			PCR plate 2	

10 mins

PCR clean up

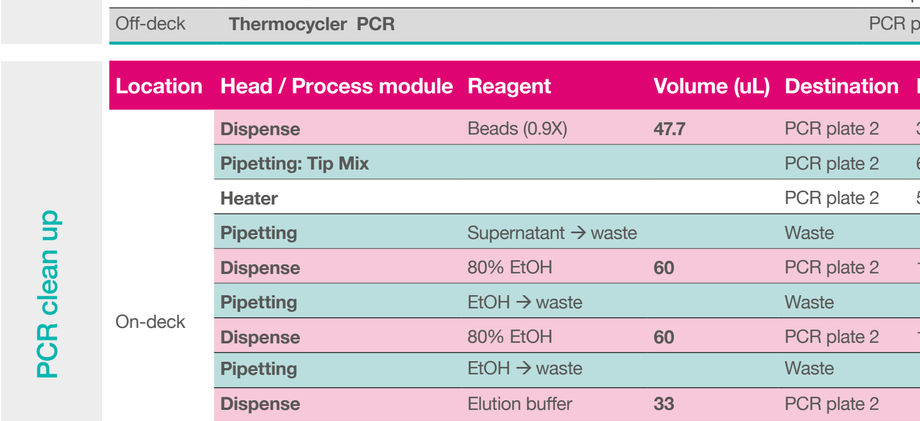
Location	Head / Process module	Reagent	Volume (uL)	Destination	Details/Dispense time	Magnet
On-deck	**Dispense**	Beads (0.9X)	**47.7**	PCR plate 2	3mins	
	Pipetting: Tip Mix			PCR plate 2	6mins	
	Heater			PCR plate 2	5mins 23°C	
	Pipetting	Supernatant → waste		Waste		On-magnet
	Dispense	80% EtOH	**60**	PCR plate 2	1min + 2min	On-magnet
	Pipetting	EtOH → waste		Waste		On-magnet
	Dispense	80% EtOH	**60**	PCR plate 2	1min + 2min	On-magnet
	Pipetting	EtOH → waste		Waste		On-magnet
	Dispense	Elution buffer	**33**	PCR plate 2		
	Pipetting: Tip mix			PCR plate 2		
	Heater			PCR plate 2	2mins 23°C	
	Pipetting	Transfer	**30**	PCR plate 3		On-magnet

40 mins

Results

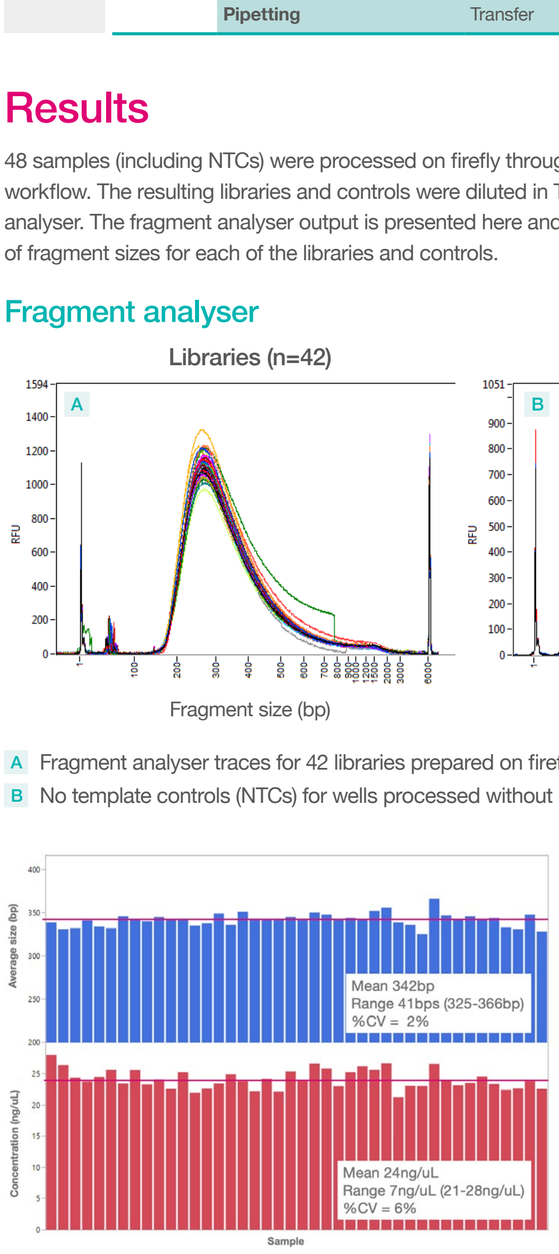
48 samples (including NTCs) were processed on firefly through the NEBNext Ultra II FS DNA workflow. The resulting libraries and controls were diluted in TE buffer then run on the fragment analyser. The fragment analyser output is presented here and is used to compare the distribution of fragment sizes for each of the libraries and controls.

Fragment analyser



A Fragment analyser traces for 42 libraries prepared on firefly - normalised to the lower marker

B No template controls (NTCs) for wells processed without input gDNA



Comparison of the average library size and concentration across the test samples. Data was taken from the fragment analyser output. The horizontal line on each graph shows the position of the mean.

Conclusions

- firefly can be used to successfully execute an NGS library preparation workflow.
- The libraries produced on firefly are uniform in concentration and the distribution of fragment sizes is uniform across the samples.
- No detectable contamination was found in the control wells which were processed without any input gDNA.

firefly’s novel combination of an air-displacement pipetting head and a positive-displacement dispense head enables all samples in a plate to be processed within seconds of each other. This uniformity in the temporal processing of samples, together with the accurate dispense performance of the firefly heads minimises sample-sample variation, making firefly well suited for the automation of NGS library preparation workflows.

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