

Fast and reliable automation of the NEBNext Ultra II FS DNA library prep kit for Illumina with firefly

Agenda

- Product introduction
- Automating NEBNext Ultra II DNA library preparation on firefly

Today's speakers



Paul Lomax

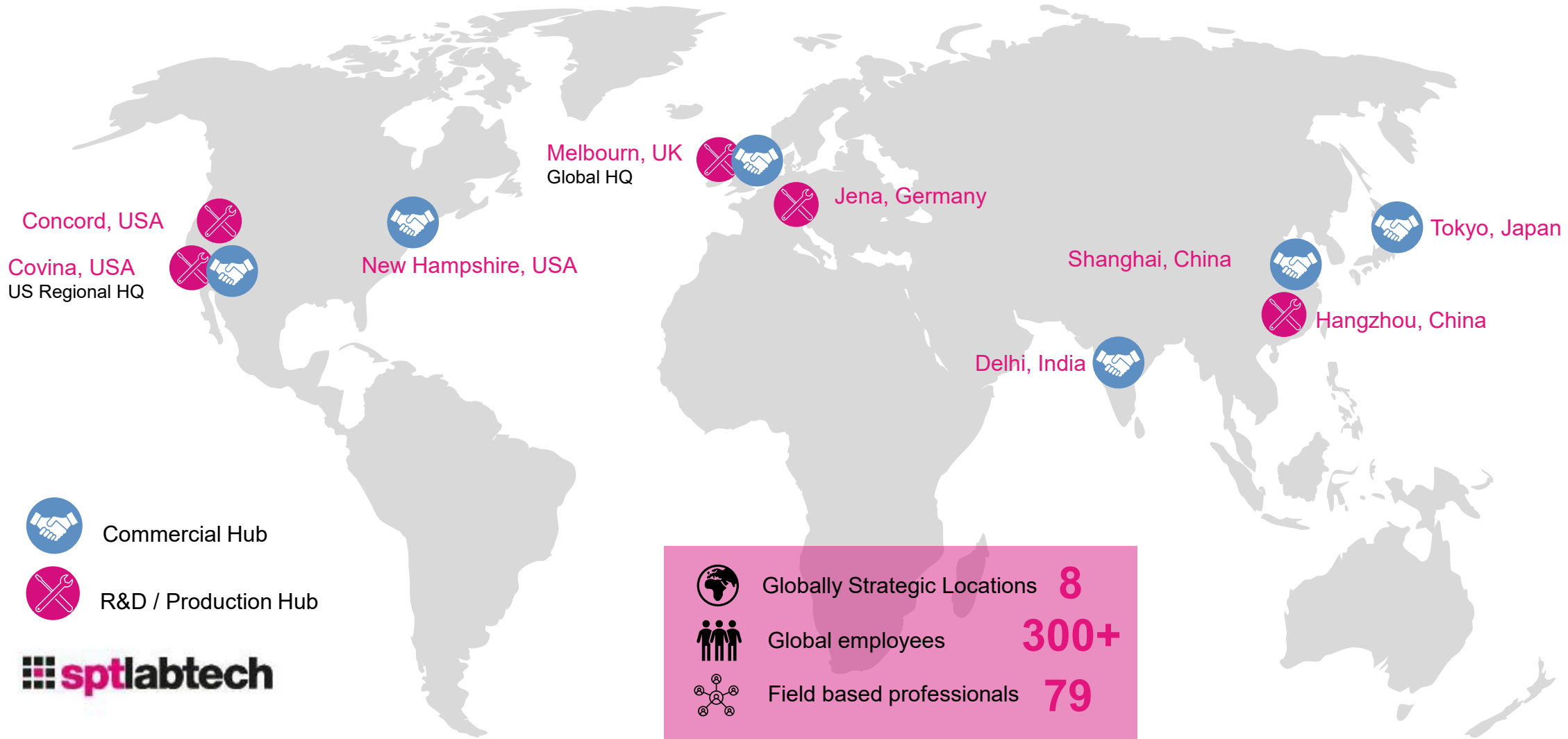
Head of Genomics | SPT Labtech



Huw Rees

Field Application Scientist | SPT Labtech

SPT Labtech global footprint





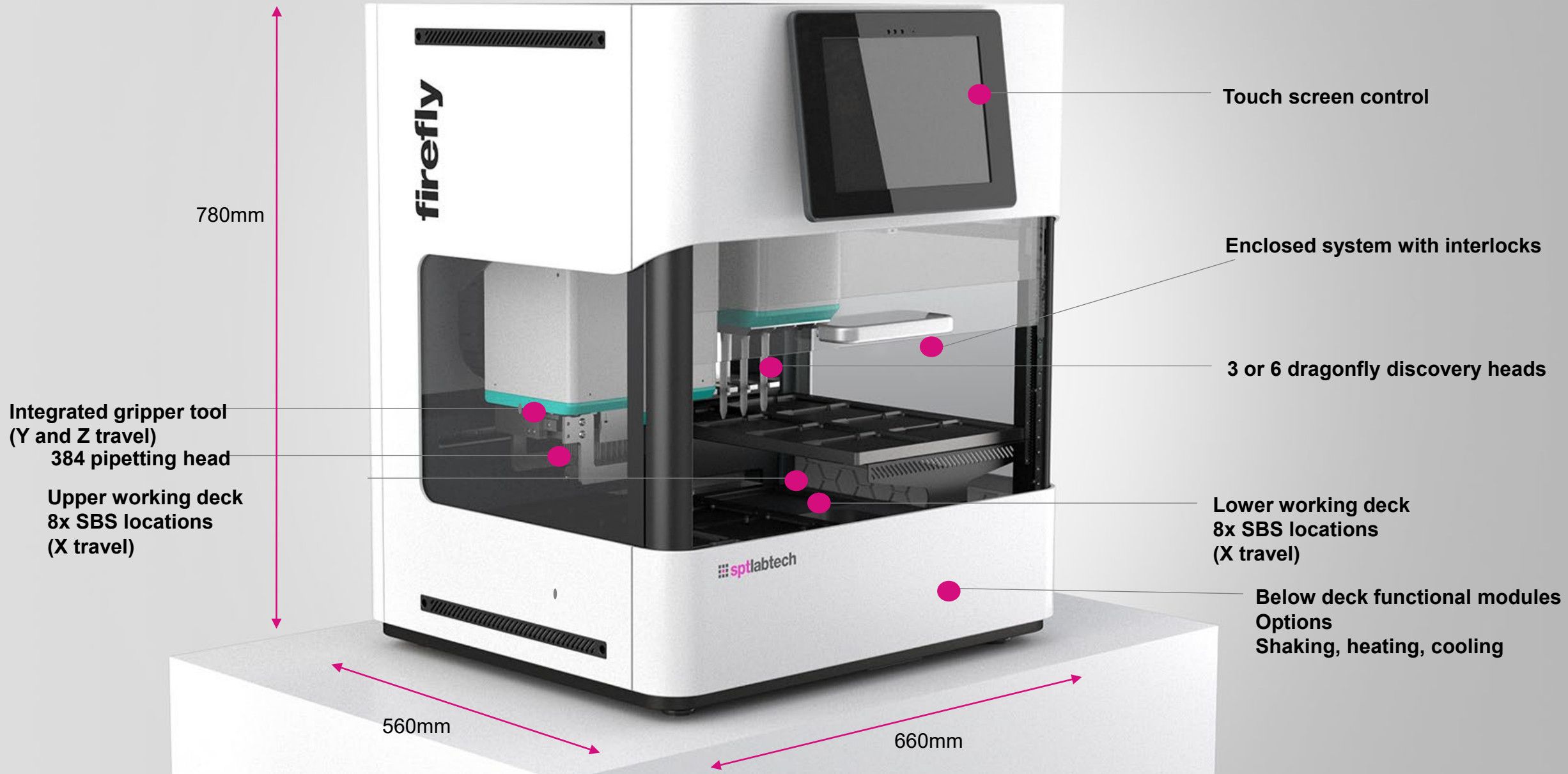
firefly

firefly

Product overview

 sptlabtech

 sptlabtech



Liquid handling capabilities

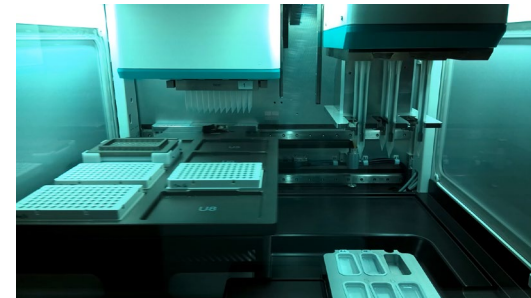
Pipetting head

- Air displacement
- 384-tip head
 - 384, 96; strips of 8 or 16
- Autoload tips
 - Cassettes of 96 or 384
 - User-loadable columns of 8 or 16
- 0.5ul to 125uL* (reduced to 100uL with filter tips)

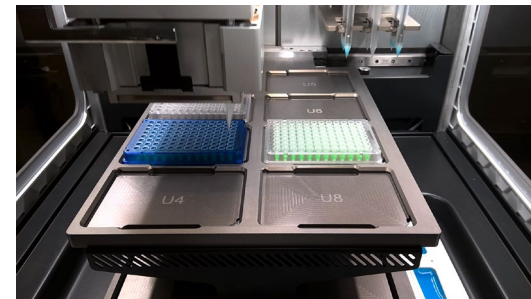
*4 in 1 adaptor in development expands to 500ul (96 and 8 strips only)



Tip change

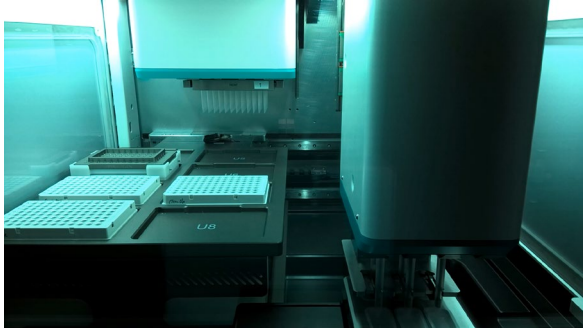


Variable mix

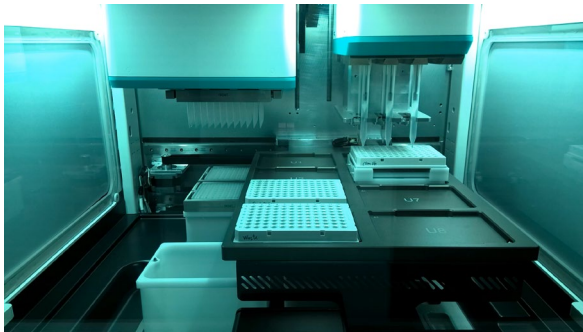


Pooling

Liquid handling capabilities



1 syringe beads



3 syringe wash

Dispensing heads

- 3 or 6 dragonfly heads
- 200nL to 4mL non-contact
- Standard dragonfly reagent reservoir options
- Disposable positive displacement tips
- Liquid class agnostic
- 96/384/1536 capable
- <1 min 5uL fill for 384 well plate (using 3 channels)

Other capabilities

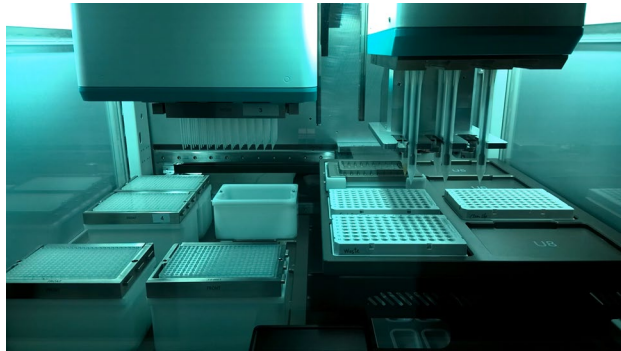
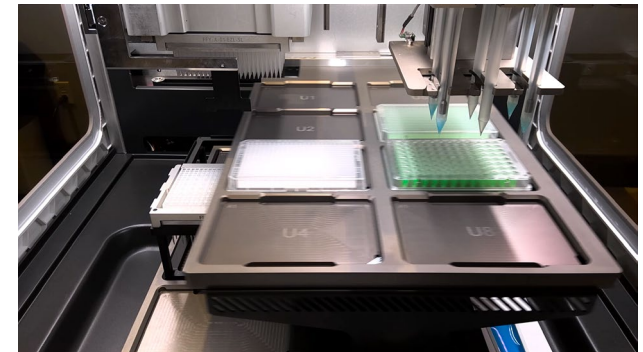


Plate move

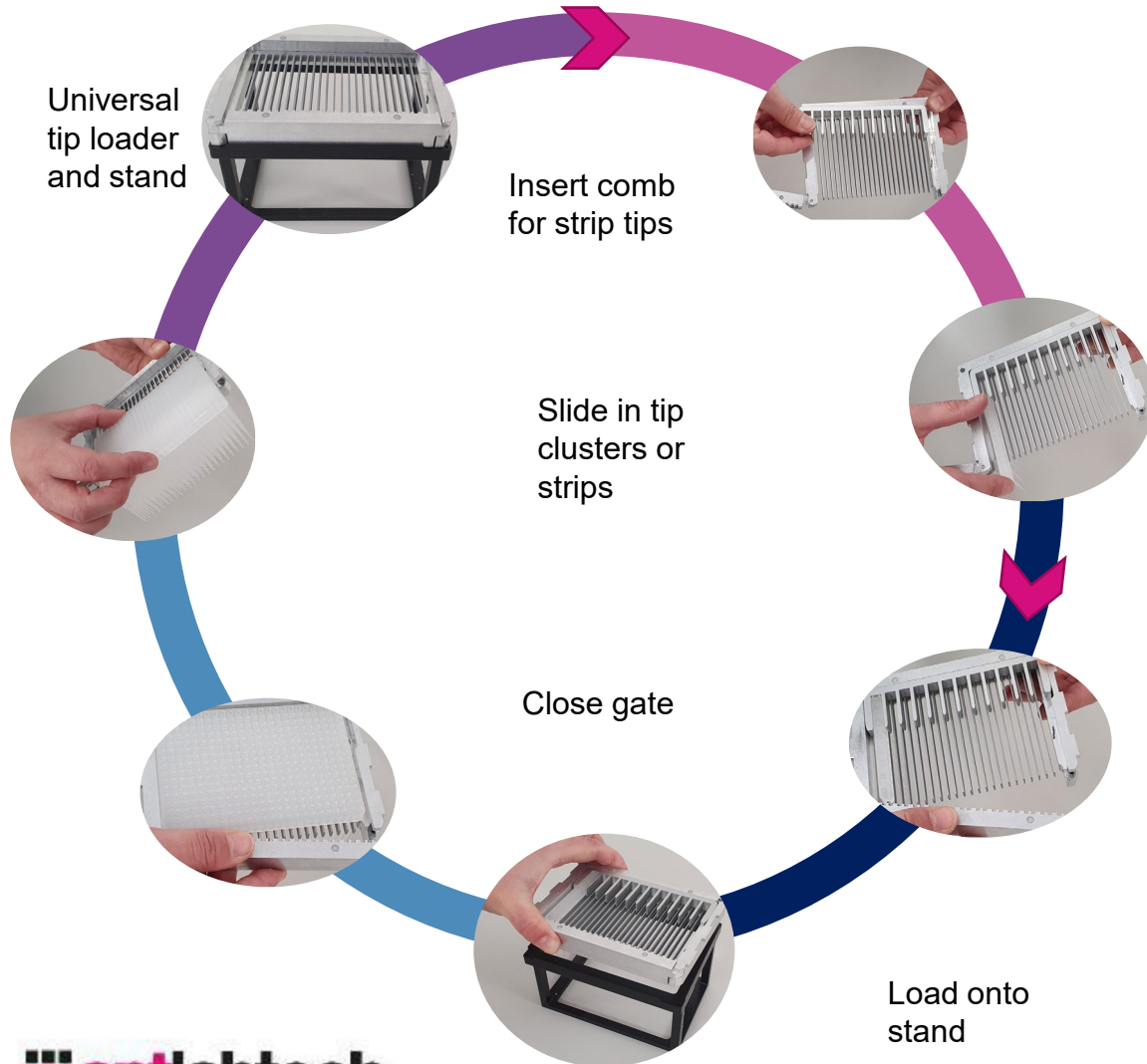


Mag block move



Move to shaker & 384 copy

Pipette head loading and tips



Tip options

- 125-096-FF-S - 125uL 96 format tips sterile
- 125-008-EZ-S - 125uL 96 format strip tips sterile (8 tips per strip)
- 125-384-EZ-S - 125uL 384 format tips sterile
- 125-016-EZ-S - 125uL 384 format strip tips sterile (16 tips per strip)
- 050-096-FF-S - 50uL 96 format tips sterile
- 050-008-EZ-S - 50uL 96 format strip tips sterile (8 tips per strip)
- 050-384-EZ-S - 50uL 384 format tips sterile
- 050-016-EZ-S - 50uL 384 format strip tips sterile (16 tips per strip)
- 125-096-FF-FS - 125uL 96 format tips filtered and sterile
- 125-008-EZ-FS - 125uL 96 format strip tips filtered and sterile (8 tips per strip)
- 125-384-EZ-FS - 125uL 384 format tips filtered and sterile
- 125-016-EZ-FS - 125uL 384 format strip tips filtered and sterile (16 tips per strip)
- 050-096-FF-FS - 50uL 96 format tips filtered and sterile
- 050-008-EZ-FS - 50uL 96 format strip tips filtered and sterile (8 tips per strip)
- 050-384-EZ-FS - 50uL 384 format tips filtered and sterile
- 050-016-EZ-FS - 50uL 384 format strip tips filtered and sterile (16 tips per strip)

application-ready accessories

- Magnetic block
 - Alapaqua Magnum FLX 96



- Shaker and incubation (Q instruments)
 - 1 x Bioshake 300 elm
 - 1 x ColdPlate for microplates
 - 1 x ColdPlate for Dispense reservoirs



Bioshake 300 elm



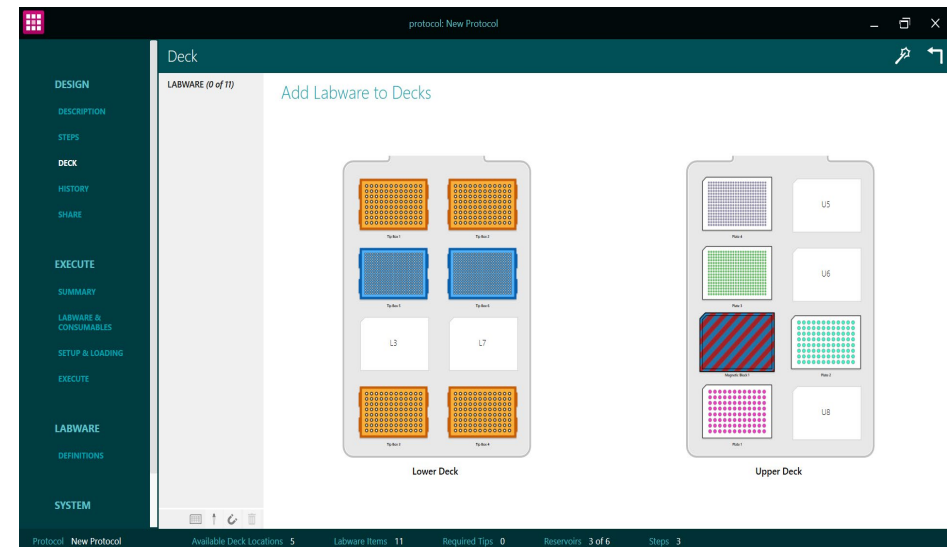
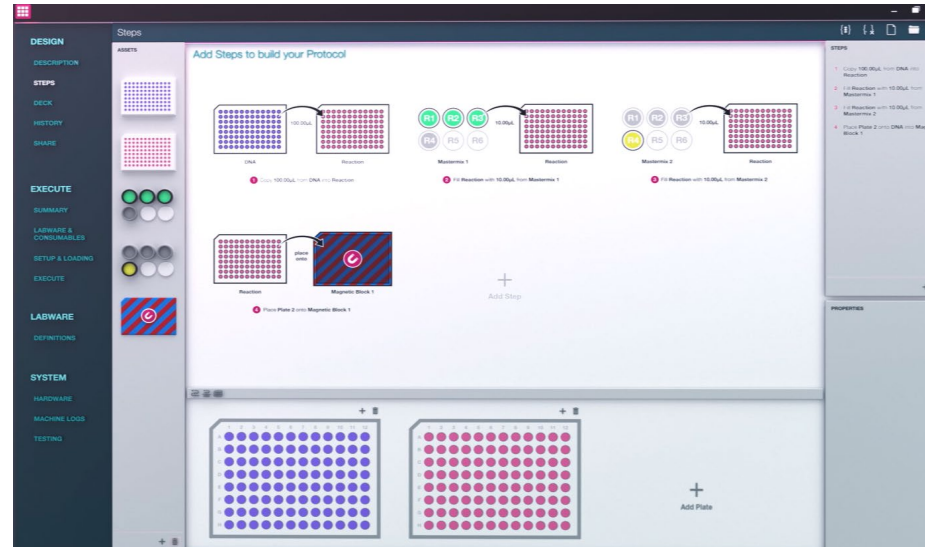
ColdPlate

- DFD accessories
Low and high-volume reservoirs, sterile tips, LVDV passive cooling block



best-in-class software

- Easy, accessible UI
- Secure log-in and audit trails
- Multiple user levels
- 'intelligent' programming
- Author on any PC
- Cloud-based sharing
- History – version management



Software key features – Design mode

Navigation pane

protocol: New Protocol

Steps

ASSETS (4)

Reaction
Plate 2 (A1:H12)

Reservoirs 1

Magnetic Block 1

1 Copy 25.00µL from DNA plate into Reaction

2 Fill Reaction with 10.00µL from Reservoirs 1

3 Place Plate 2 onto a stackable item

PROPERTIES

Description

Source DNA plate

Destination Reaction

Liquid Class None

Volume (µL) 25.00

ASPIRATE

Speed (µL/sec) 15.00

Start Height (%) 50.00

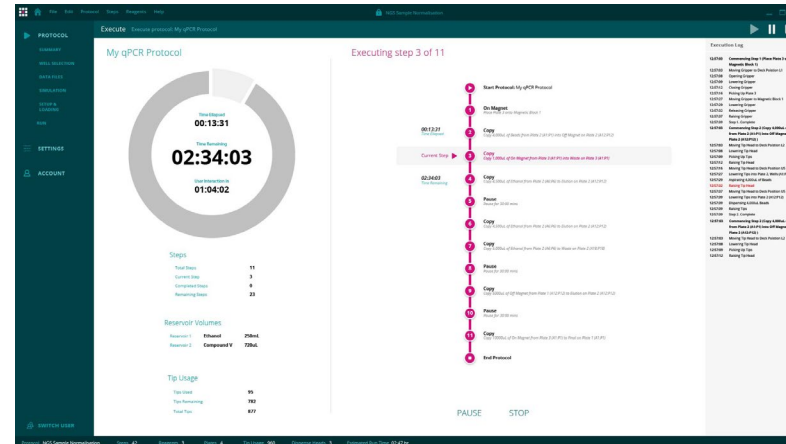
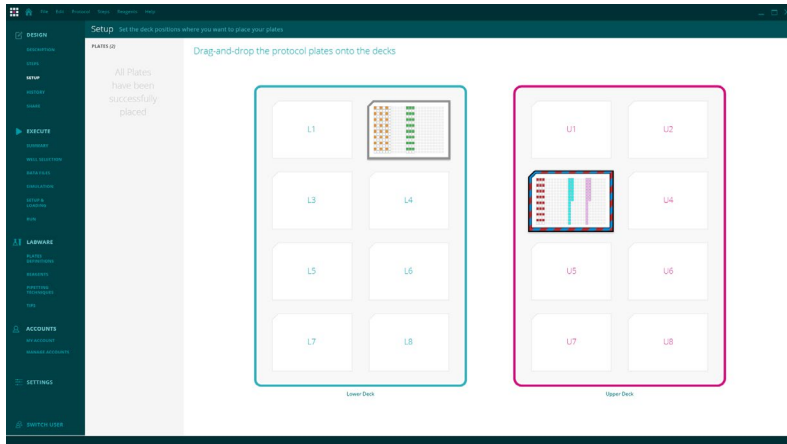
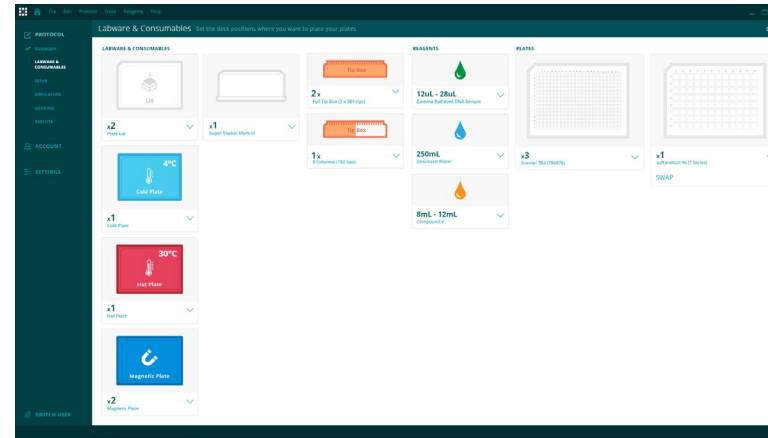
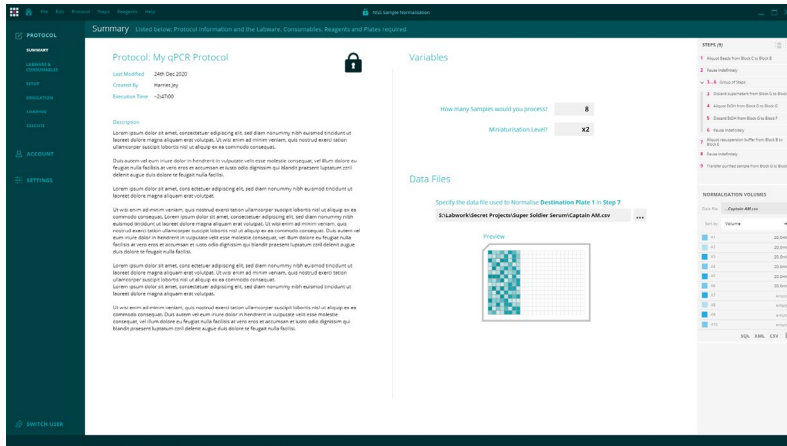
Protocol New Protocol

Available Deck Locations 16 Labware Items 3 Required Tips 0 Reservoirs 3 of 6 Steps 3

Parameter control

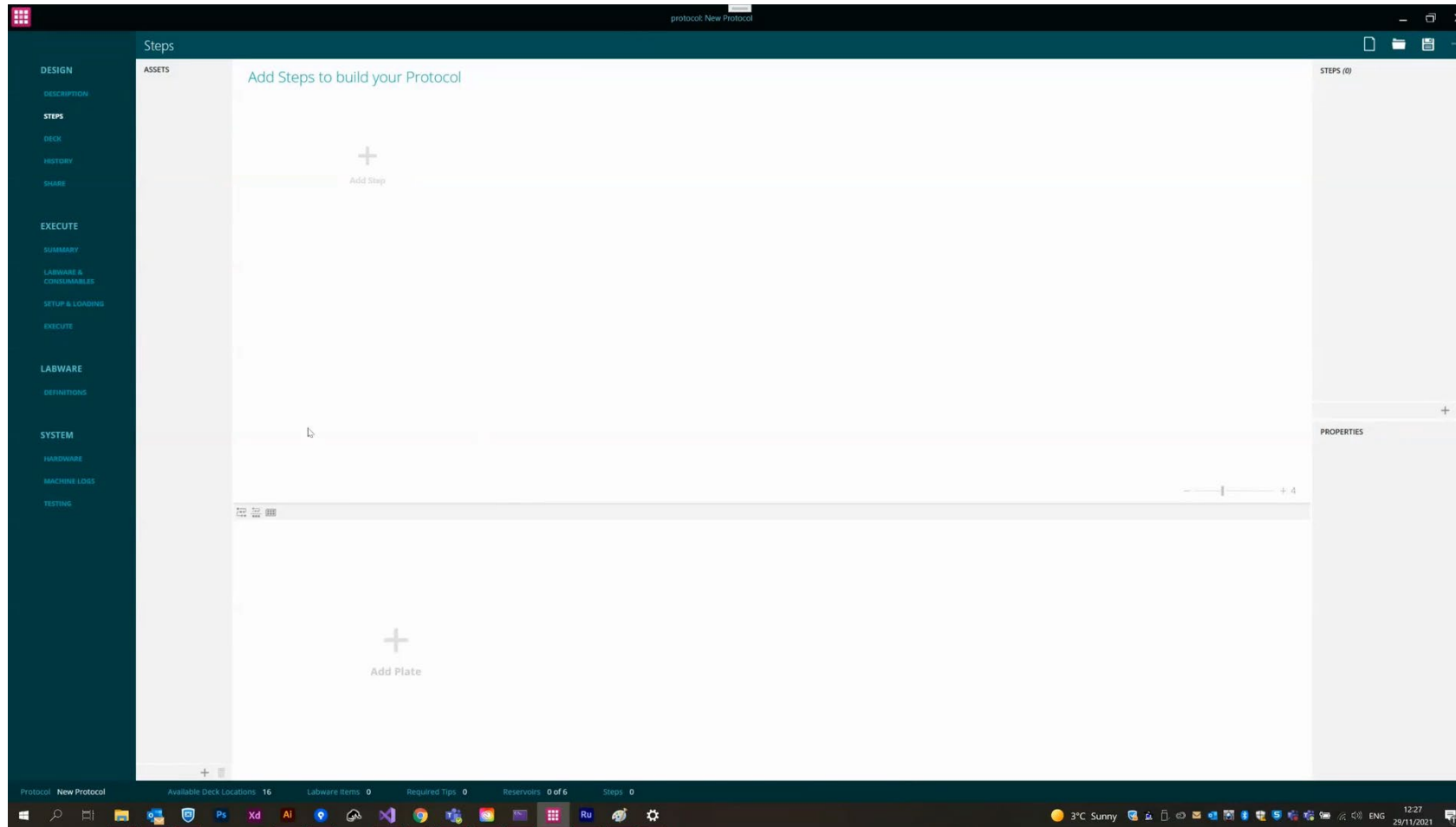
Protocol designer

Software key features – Execute mode



1. Overview and variables
2. “Shopping list”
3. Runtime loading wizard
4. Runtime dashboard

Software demo



Peer to peer cloud



deepen collaboration &
accelerate research

- Greater control
 - Author protocols anywhere
 - Share protocols across systems
 - Ensure consistency
 - Greater security
- Deepen collaboration
 - Site wide
 - Intra company
 - Globally
 - You decide – how much, how little
- Simple sharing
 - Protocols
 - Labware
 - Liquid classes

Current applications

- NEBNext Ultra II DNA
- NEBNext Ultra II FS DNA
- NEBNext Ultra II RNA
- Illumina DNA Prep
- Library of new applications to follow:
 - 1 per month from Takara, Thermo, Kapa, Roche, ...
 - Customer cloud sharing of custom protocols



The logo for Illumina, consisting of the word "illumina" in a lowercase, black, sans-serif font, with a registered trademark symbol (®) to its upper right.

bundles and options

- firefly 3 or firefly 6
 - Identical apart from 3 or 6 dispense heads
- Genomics options kit
 - 1 x Bioshake 200 elm
 - 2 x ColdPlate (1 for microplates, 1 for dispenser reservoirs)
 - 2 x adapter plates for ColdPlate
- Consumables starter kit
- Multi-level Reliance service contracts available

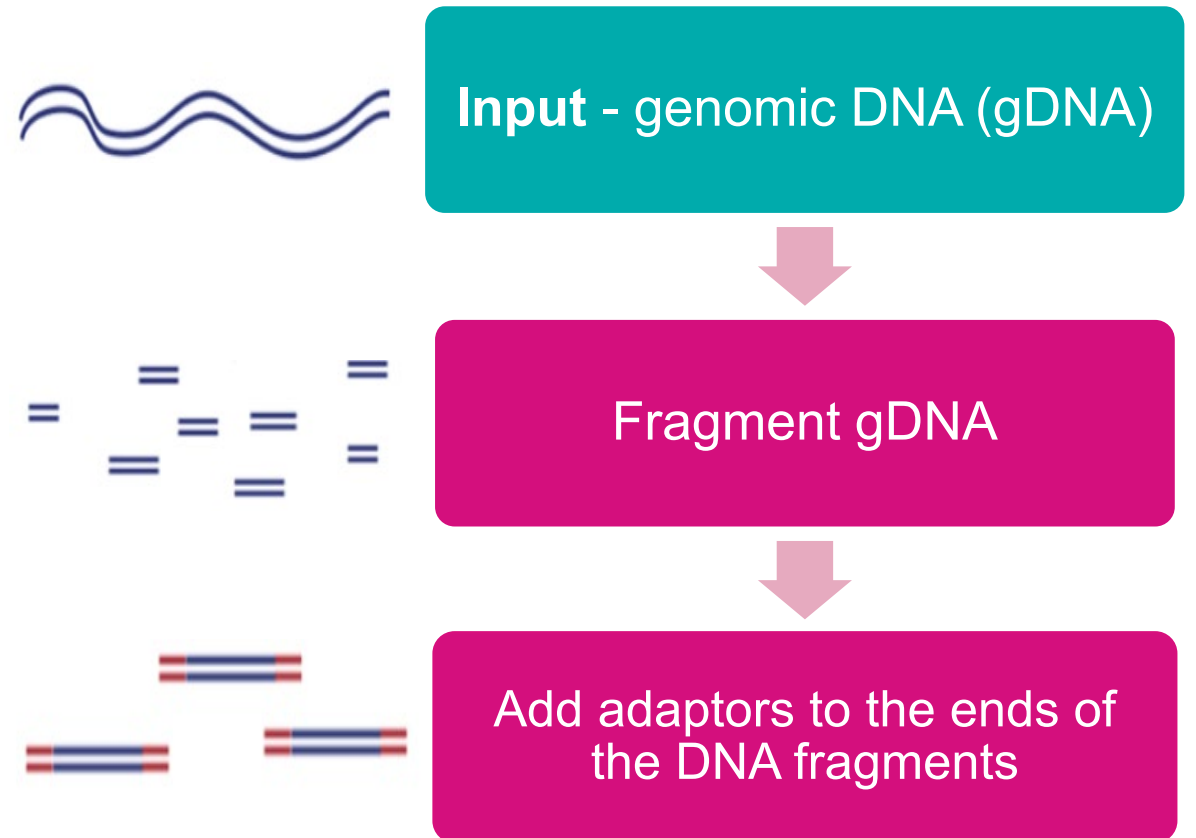
Key benefits summary

- Combined functionality of pipetting, dispensing, incubating and **shaking overcomes the requirement for multiple instruments** saves cost and space, reduces oversight and training burden, and eliminates equipment redundancies.
- **firefly is dedicated to NGS library prep** so addresses the workflow problems specifically relevant to this activity. Having a dedicated **instrument saves time, ensures accuracy and delivers a better performance.**
- The **compact footprint** with vertical multi-layered system **saves precious lab space** and multiplies how many plates you can be filling at once.
- **Sets a new standard** for intuitive, collaborative liquid handling software providing easy access to new applications and protocols from researchers through a cloud-based network.
- **Intuitive, easy-to-use** instrument with attractive streamlined design – making it a desirable asset for a laboratory.

Introduction

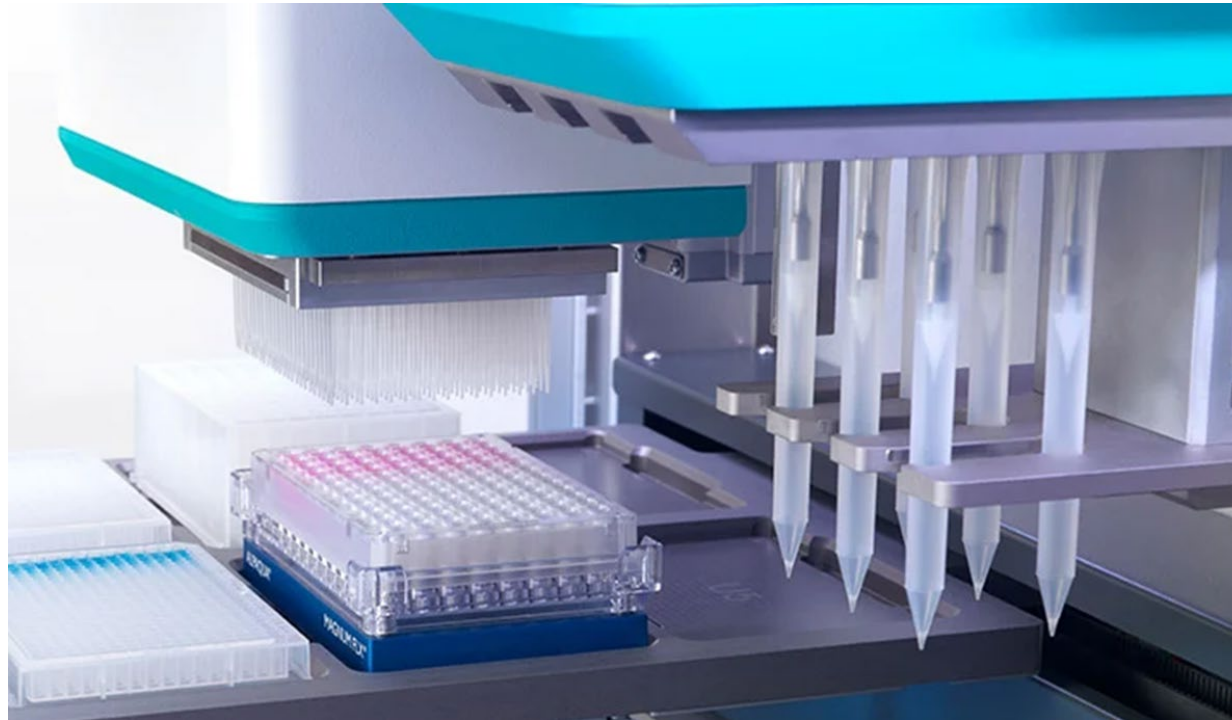
Introduction

- 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



Introduction

- 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



Introduction

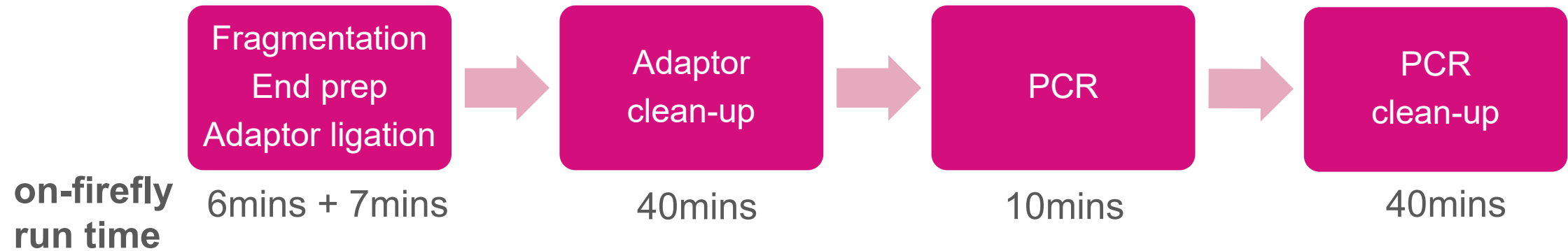
firefly consists of:

- 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

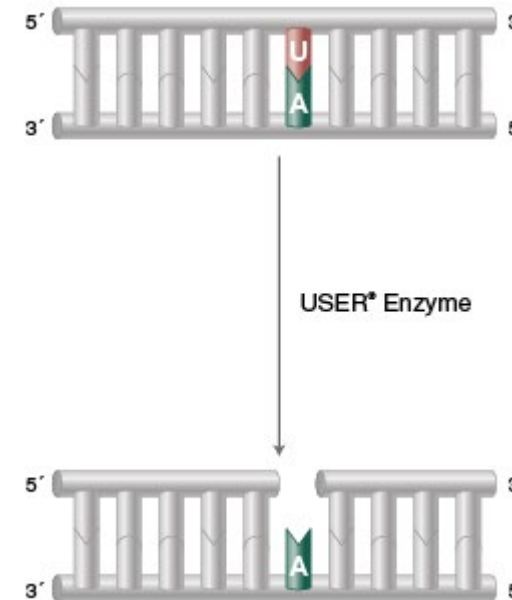
Method

- 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
 - 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

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

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

Steps

Steps

- The following tables 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

	Dispense head
	Pipetting head
	Process module
	Off-deck

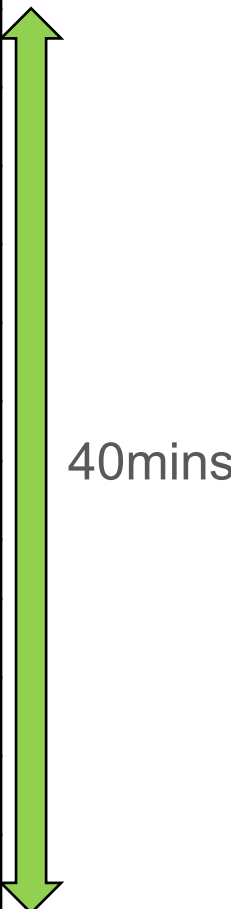
Fragmentation | End-prep | Adaptor ligation

Location	Head / Process module	Reagent	Volume (uL)	Destination	Details / Dispense time	
On-deck	Dispense	FS enzyme+ buffer	9	PCR plate 1	1.5min	6mins
	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	7mins
	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	

Steps

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	



Steps

PCR

Location	Head	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	

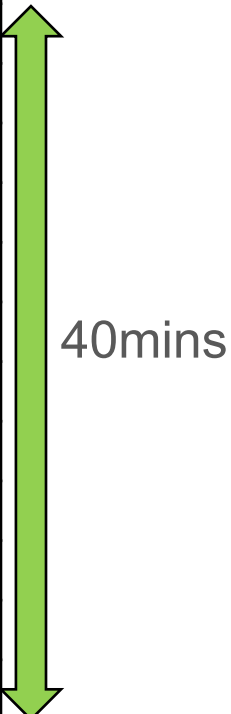


10mins

Steps

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



Results

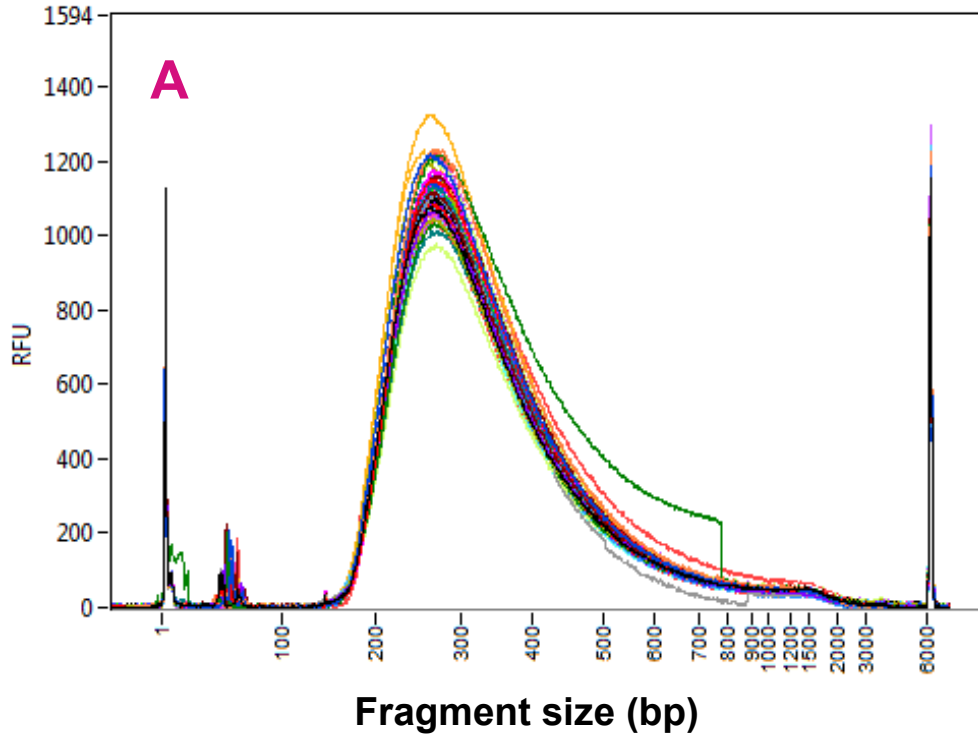
Results

- 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

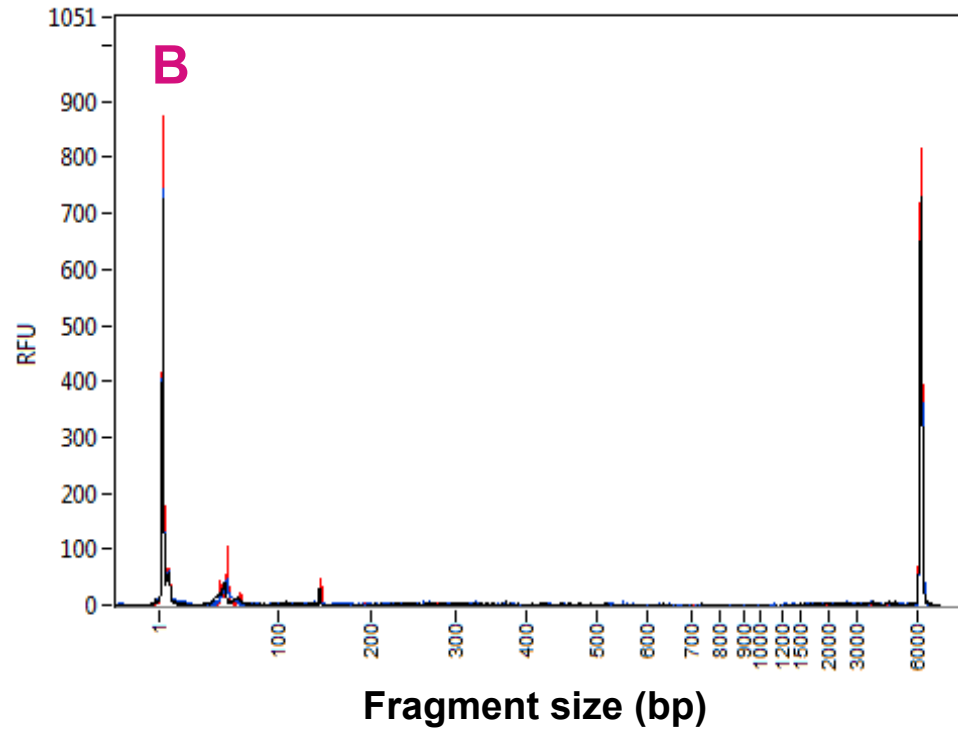
Results

Fragment analyser

■ Libraries (n=42)



■ NTCs



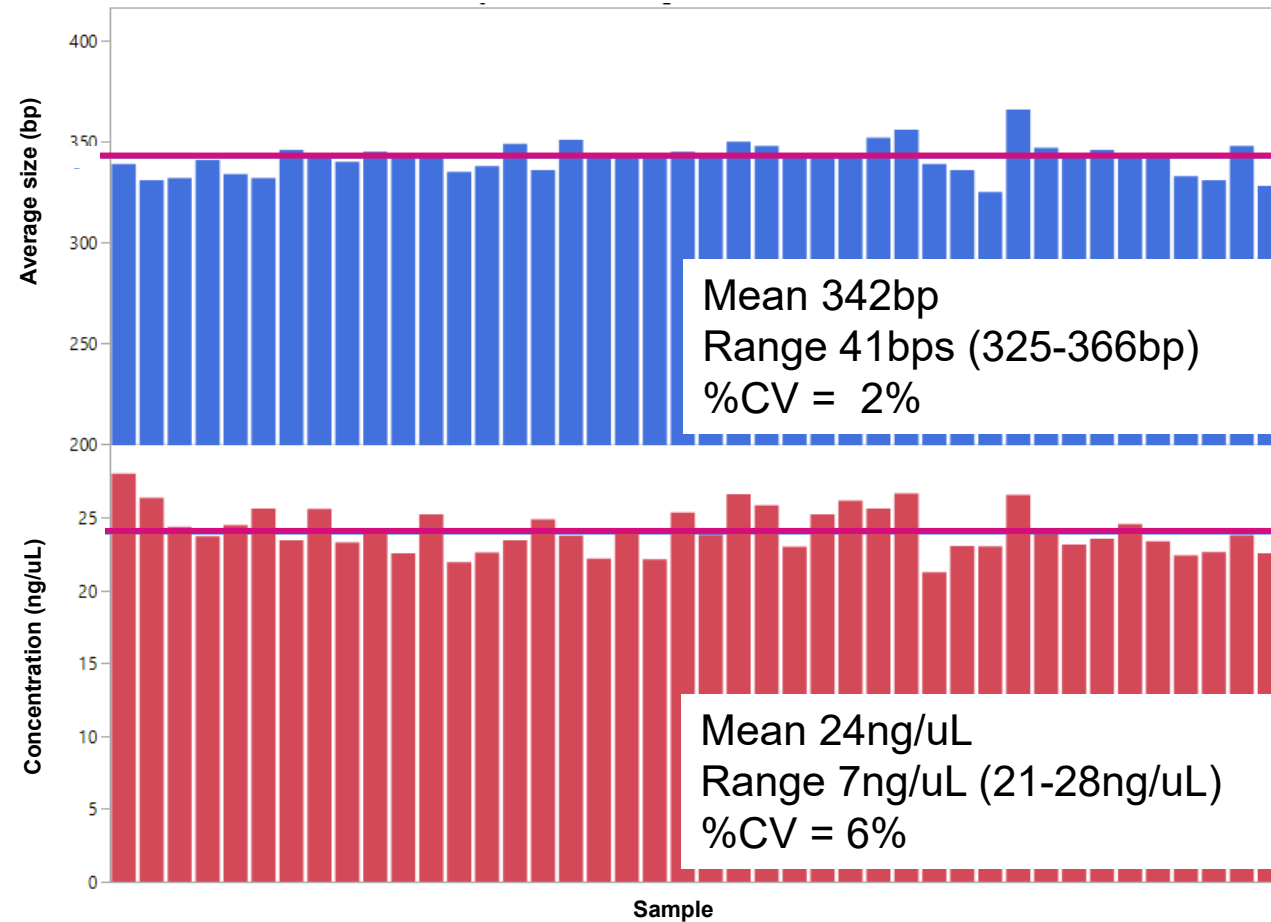
A: Fragment analyser traces for 42 libraries prepared on firefly - normalised to the lower marker

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

Results

Fragment analyser

- 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



Conclusions

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.



Conclusions

Just the beginning...



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