

# Next-gen Sequencing

Automated solutions for library preparation



**HAMILTON**®



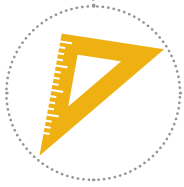
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# Precision & Accuracy

## Handle your valuable liquids with confidence

When it comes to your valuable samples and reagents, Hamilton's leading pipetting technology ensures extensive process control and the highest degree of reliability.

With precise air displacement pipetting technology, and integrated real-time pressure monitoring of each pipetting step, our platforms offer unmatched performance for low volume pipetting and sample handling. Every step in the library preparation workflow is analyzed, verifying transfer volumes. Sample and reagent quantity is measured by liquid level detection and end users can be notified of any inconsistencies such as insufficient liquid, clots or air bubbles.

This capability ensures methods are executed reliably without compromising sample processing.

The highest performance requires the highest technology!

- ▶ **More for less : increased sample recovery yields compared to manual methods**
- ▶ **Reagent savings: less dead volume, reduced reagent waste**
- ▶ **Consistently high reads on target**





# Traceability & Tracking

Everything under your control



Hamilton liquid handling workstations feature state-of-the-art security while focusing on complete process control and robustness.

All platforms ensure full sample tracking during every step of your workflow; primary samples and destination plate IDs are tracked and stored. Our platforms have extensive file handling capabilities to suit the end user's data management infrastructure, including LIMS integration.

Have complete confidence in your results! Each step is monitored, ensuring proactive error handling for maximum sample safety.

- ▶ **1D and 2D barcode reading for primary samples and destination plates**
- ▶ **Sample tracking data is fully traceable; internal database maintains real-time storage of all process data and sensitive information**
- ▶ **Unlimited interfacing options with external LIMS, thus no more manual data transfers**





# Modularity & Scalability

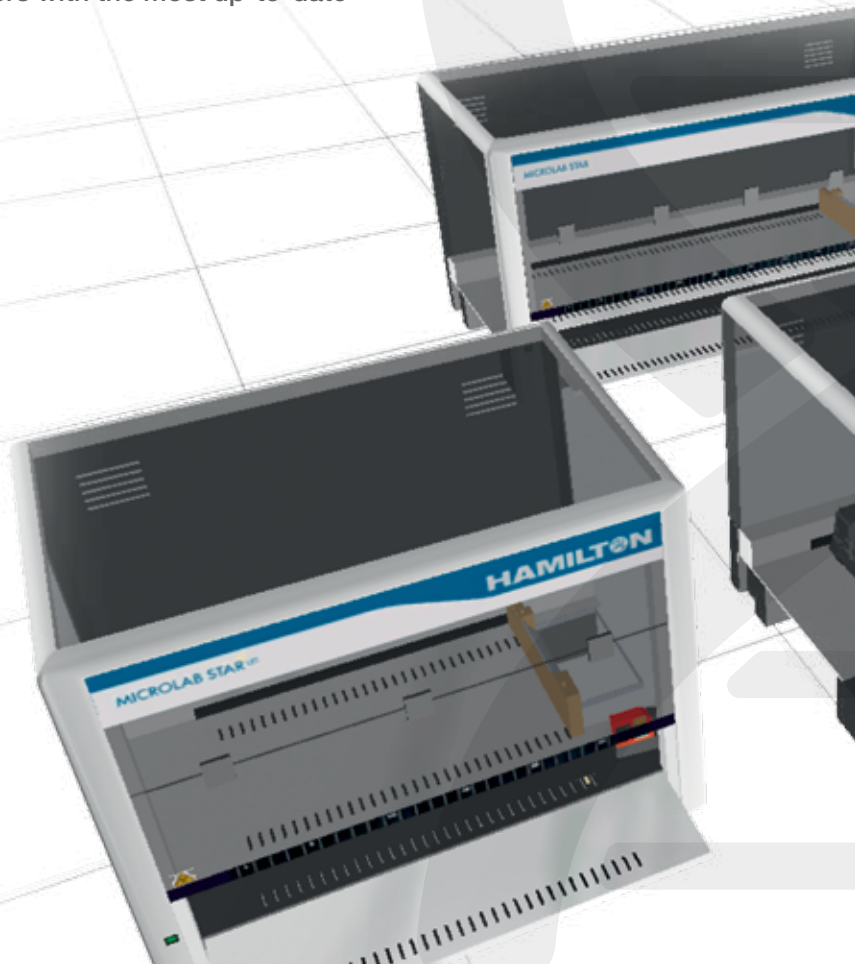
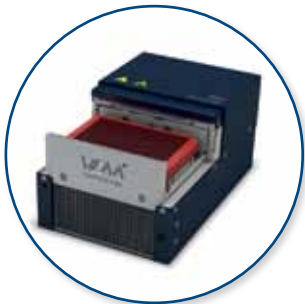
## New kits, new protocols - same workstation

All Hamilton platforms are developed to evolve in parallel with your scientific needs; new kits and new workflows are released on a regular basis in the NGS field.

The modular design of Hamilton's liquid handling systems allows them to adapt to the end user's changing requirements such as accommodating an increase in sample throughput and walkaway time, or adding extra functionality with deck accessories and pipetting tools.

Many upgrades can be performed in field making our platforms future-proof and the scope for new assay development achievable.

- ▶ **More throughput:** upgrade with more pipetting channels or add a multiprobe head with 96 or 384 parallel pipetting channels
- ▶ **More functionality:** upgrade with on-deck thermal cycling or cooled positions for samples and reagents
- ▶ **More methods:** by collaborating with all major NGS kit providers, we can provide existing customers with the most up-to-date protocols and methods



# Flexibility & Versatility

## The right platform for every application

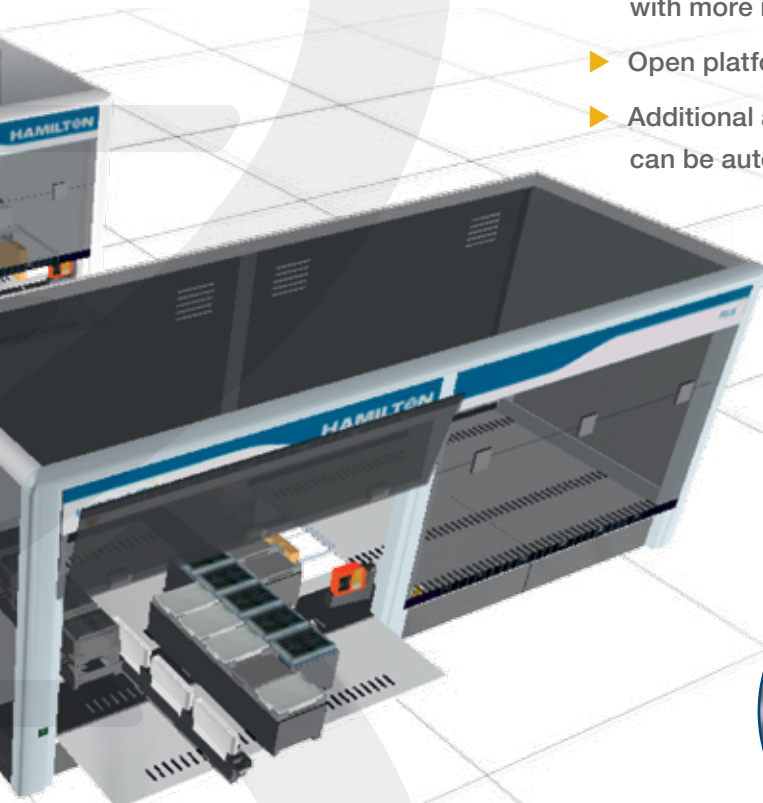


With a wide range of supplier validated and customer approved protocols, you will benefit from pretested automated workflows.

Methods can be installed and implemented on your platform using a simple “copy and paste.” At the same time, all Hamilton systems are open platforms to ensure the highest degree of flexibility and versatility. Other protocols, such as nucleic acid extraction (vacuum filtration or magnetic bead based), PCR setup or mother/daughter plate replication can be programmed via the flexible Venus software and run on a routine basis.

Peripheral devices, such as plate readers, thermal cyclers or plate sealers, can be integrated and fully controlled by the same, central user interface. There are no application limitations, today or in the future!

- ▶ **More than 15 NGS workflows available now, with more regularly being released**
- ▶ **Open platform: use any kit and any protocol**
- ▶ **Additional assay scope: downstream processes can be automated on the same platform**



# Introduction

The following section illustrates a brief description of the main next-generation sequencing library preparation methods available from Hamilton.

As a leading player in the NGS automation field, Hamilton continuously develops new methods which will be available for all Hamilton users.

To find out more about the latest updates, please contact your Hamilton representative.

The **Next-gen sequencing methods** presented in this overview are categorized as the following:



**Qualified method:** the method was tested and approved by the kit manufacturer



**Approved method:** the method was tested and approved by an independent scientific body or a customer



**Verified method:** the method was tested and verified internally by Hamilton

In describing the automation of each method, we represented the **most efficient robotic configurations** for automation of the protocol.

A graphical representation of the **instrument deck layout** is convenient to understand where samples, reagents and all consumables are placed onto the deck.

The “**walkaway batch size**” indicates the number of samples the system can run autonomously, without any user intervention.

The block diagram, which represents the **complete workflow** of the method, shows the steps divided by color:



Blue blocks illustrate steps performed at room temperature



Red blocks illustrate steps involving incubation or thermal cycling



Grey blocks illustrate steps performed off-line



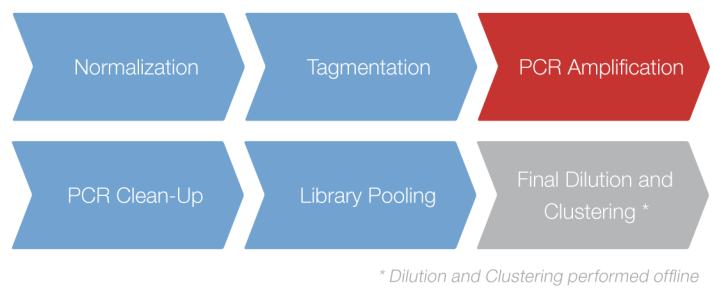




# Illumina Nextera XT library preparation

## Description and Workflow

High throughput, automation friendly and rapid preparation of sequencing-ready libraries for small genomes (bacteria, archaea, viruses), amplicons, and plasmids. With the full recommended configuration, 96 libraries can be prepared and pooled in 6 hours with no user intervention.

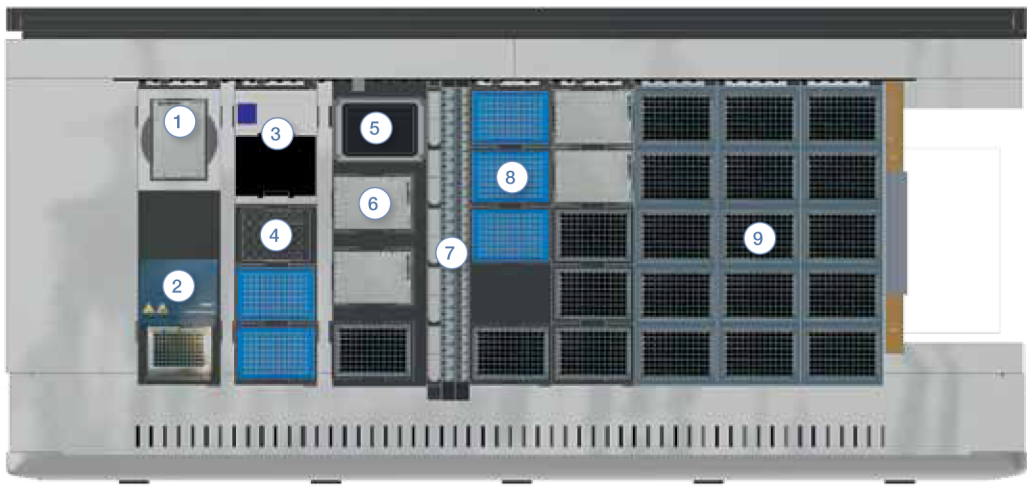


“ We have found our Hamilton STAR to be reliable, robust and adaptable to our sequencing protocol needs. I am particularly keen on the easy-to-use and powerful programming interface and excellent support from the company.”

Dr. Nick Loman, Ph.D  
 Independent Research Fellow, MicrobesNG, University of Birmingham

”

## Deck Layout



- 1 Turntable
- 2 On Deck Thermal Cycler
- 3 Cooling module
- 4 Reagents
- 5 Hamilton Heater Shaker
- 6 Magnet position
- 7 Reagent troughs & Reagent tubes
- 8 PCR plate positions
- 9 Tips

## Recommended Platforms

Platform	Pipetting Channels	96 MPH	Batch size	ODTC **
STARlet ML *	4	No	48 samples ***	No
STAR ML *	8	No	96 samples ***	No
STAR ML *	8	No	96 samples	Yes

\*\*\* Incubations and PCR offline, reloading of tips necessary, plate needs to be manually rotated once during the process. \* ML: Manual Load \*\* On Deck Thermal Cycler



# Illumina SeqLab TruSeq Nano DNA

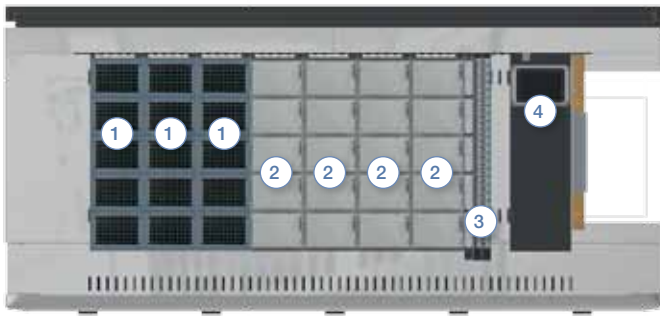
## Description and Workflow

This kit prepares up to 96 pooled, indexed paired-end libraries of genomic DNA (gDNA) for subsequent cluster generation and DNA sequencing in HiSeq X sequencing platforms. This method does use a PCR step.

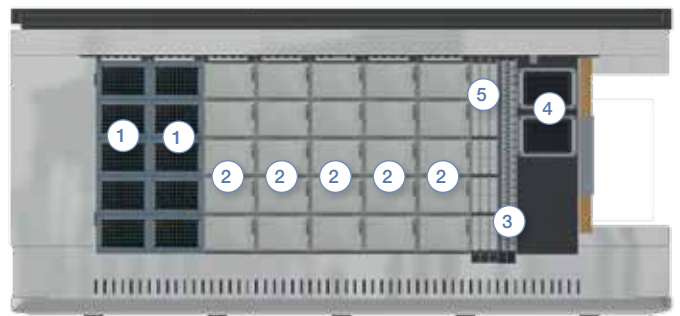


\* Incubations are performed on Hamilton Heater Shakers (HHS) set at different temperatures. PCR amplification is performed off line. Optional on-line Thermal Cycling with ODTc.  
\*\* Library Validation is performed off-line on 3<sup>rd</sup> party devices.

## Deck Layout



Pre-PCR STAR for HiSeq X



Post-PCR STAR for HiSeq X

- ① Tip Carriers
- ② Plate Carriers
- ③ Reagent Carriers
- ④ HHS (fixed carrier)\*

- ① Tip Carriers
- ② Plate Carriers
- ③ Reagent Carriers
- ④ HHS (fixed carrier)\*
- ⑤ Strip Tube

## Recommended Platforms

Platform	Pipetting Channels	96 MPH	Batch size	ODTC *
STAR	8	Yes	96 samples	No

\* On Deck Thermal Cycler

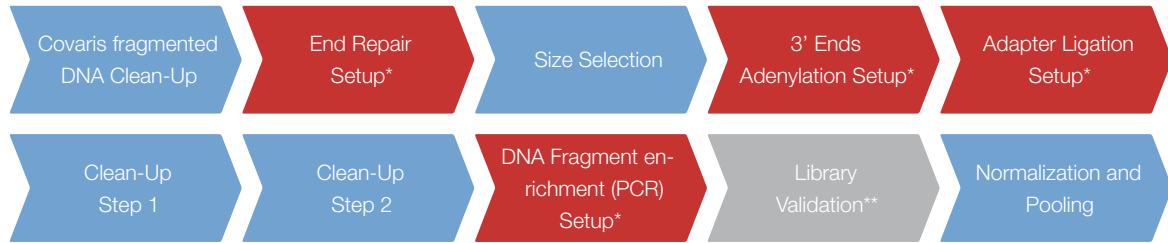




# Illumina SeqLab TruSeq PCR free DNA

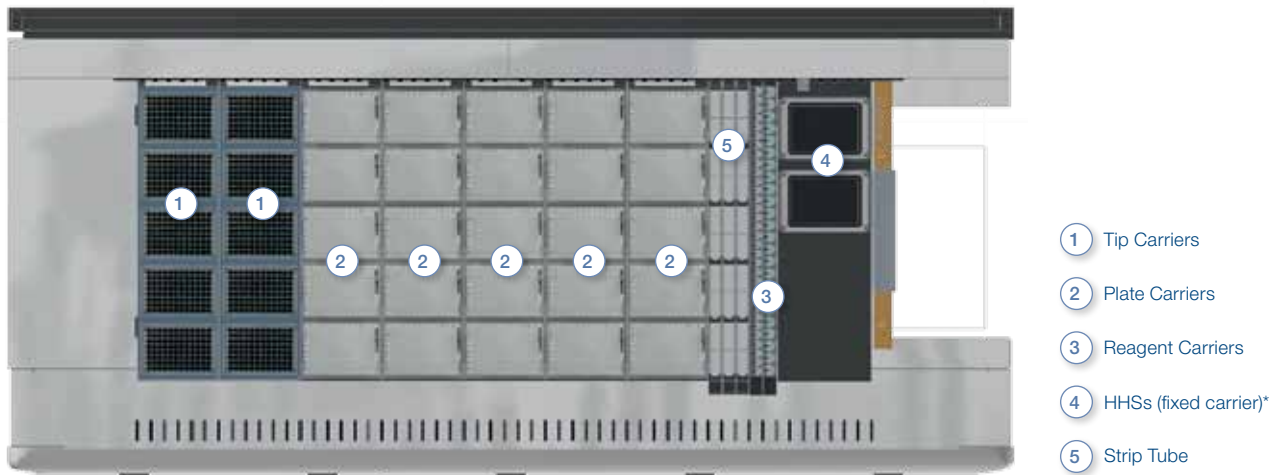
## Description and Workflow

This kit prepares up to 96 pooled, indexed paired-end libraries of genomic DNA (gDNA) for subsequent cluster generation and DNA sequencing on HiSeq X sequencing platforms. This method does not use PCR.



\* Incubations are performed on Hamilton Heater Shakers (HHS) set at different temperatures. PCR amplification is performed off line. Optional on-line Thermal Cycling with ODTc.  
\*\* Library Validation is performed off-line on 3<sup>rd</sup> party devices.

## Deck Layout



## Recommended Platforms

Platform	Pipetting Channels	96 MPH	Batch size	ODTC *
STAR	8	Yes	96 samples	No

\* On Deck Thermal Cycler



# Illumina TruSeq Nano DNA

## Description and Workflow

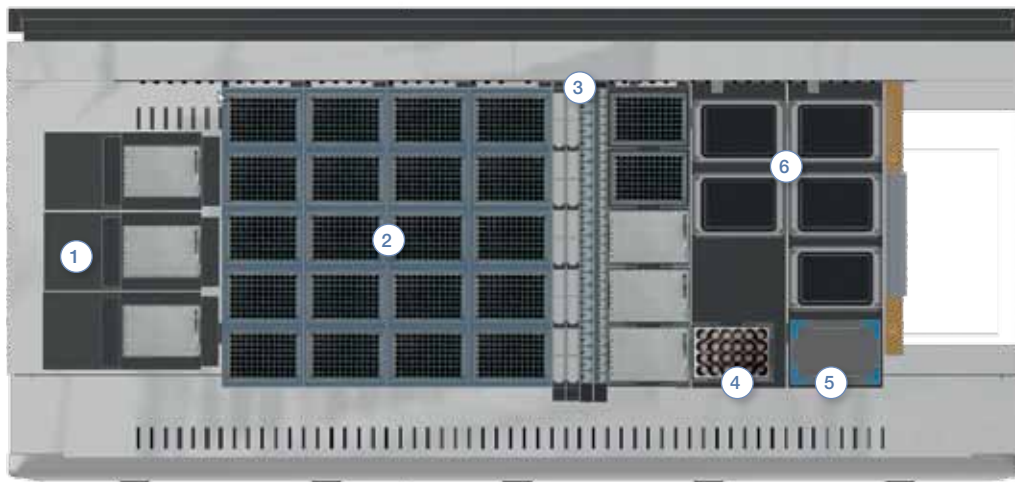
This kit prepares up to 96 pooled, indexed paired-end libraries of genomic DNA (gDNA) for subsequent cluster generation and DNA sequencing.

The goal of this protocol is to add adapter sequences onto the ends of DNA fragments to generate indexed single read or paired-end sequencing libraries. The method uses PCR steps.



\* Incubations are performed on Hamilton Heater Shakers (HHS) set at different temperatures. PCR amplification is performed off line. Optional on-line Thermal Cycling with ODTc.  
\*\* Library Validation is performed off-line on 3<sup>rd</sup> party devices.

## Deck Layout



- 1 Plate Stackers
- 2 Tips
- 3 Reagents
- 4 Magnet
- 5 CPAC
- 6 Hamilton Heater Shakers (5)

## Recommended Platforms

Platform	Pipetting Channels	96 MPH	Batch size	ODTC *
STARlet	8	No	48 samples	No
STAR	8	No/Yes	96 samples	Yes

\* On Deck Thermal Cycler



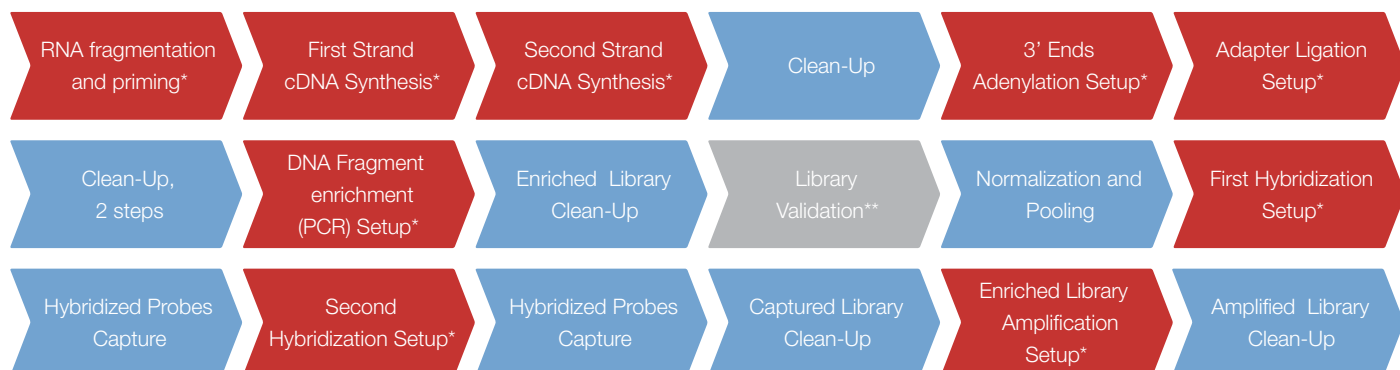




# Illumina TruSeq RNA Access

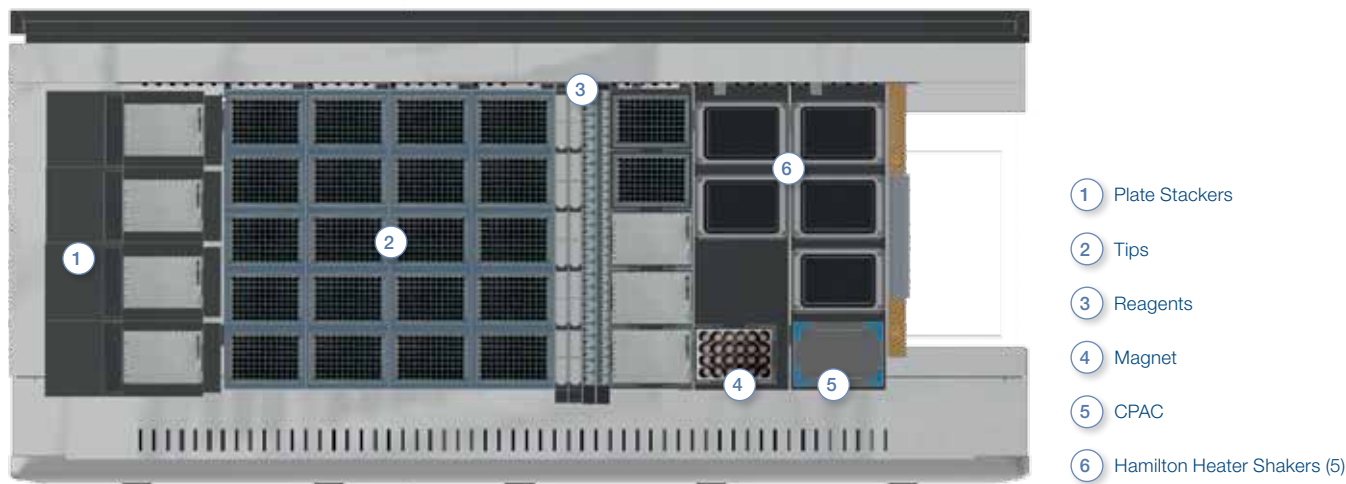
## Description and Workflow

This protocol converts total RNA into a library of template molecules of known strand origin and then captures the coding regions of the transcriptome. The resulting library is suitable for subsequent cluster generation and sequencing. The RNA is fragmented into small pieces using divalent cations under elevated temperature. cDNA is generated from the cleaved RNA fragments using random priming during first and second strand synthesis and sequencing adapters are ligated to the resulting double-stranded cDNA fragments. The coding regions of the transcriptome are then captured from this library using sequence-specific probes to create the final library.



\* Incubations are performed on Hamilton Heater Shakers (HHS) set at different temperatures. PCR amplification is performed off line. Optional on-line Thermal Cycling with ODTc.  
 \*\* Library Validation is performed off-line on 3<sup>rd</sup> party devices.

## Deck Layout



## Recommended Platforms

Platform	Pipetting Channels	96 MPH	Batch size	ODTC *
STARlet	8	No	48 samples	No
STAR	8	No/Yes	96 samples	Yes

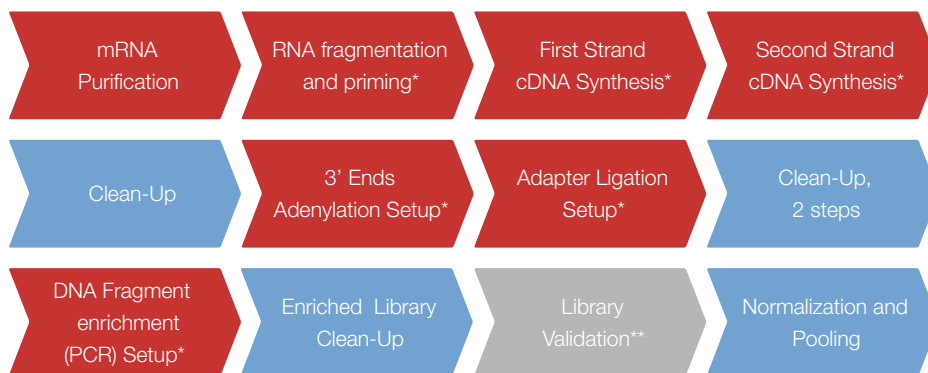
\* On Deck Thermal Cycler



# Illumina TruSeq Stranded mRNA

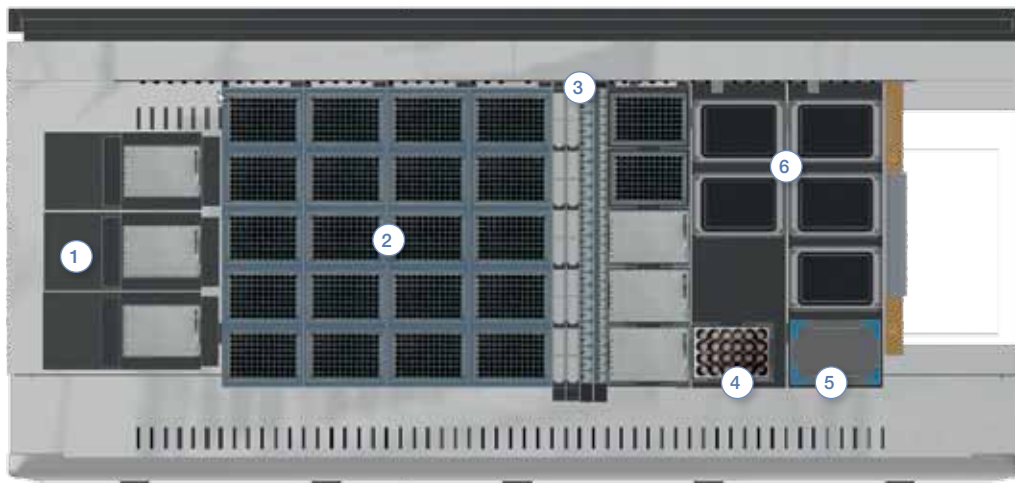
## Description and Workflow

This protocol converts the mRNA from total RNA into a library of template molecules of known strand origin. The library is suitable for subsequent cluster generation and DNA sequencing.



\* Incubations are performed on Hamilton Heater Shakers (HHS) set at different temperatures. PCR amplification is performed off-line. Optional on-line Thermal Cycling with ODTC.  
 \*\* Library Validation is performed off-line on 3<sup>rd</sup> party devices.

## Deck Layout



- 1 Plate Stackers
- 2 Tips
- 3 Reagents
- 4 Magnet
- 5 CPAC
- 6 Hamilton Heater Shakers (5)

## Recommended Platforms

Platform	Pipetting Channels	96 MPH	Batch size	ODTC *
STARlet	8	No	48 samples	No
STAR	8	No/Yes	96 samples	Yes

\* On Deck Thermal Cycler

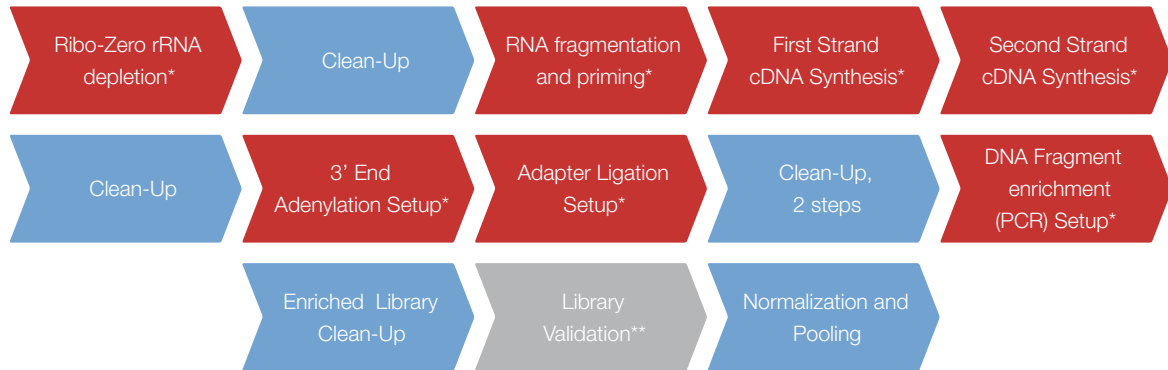




# Illumina TruSeq Stranded Total RNA

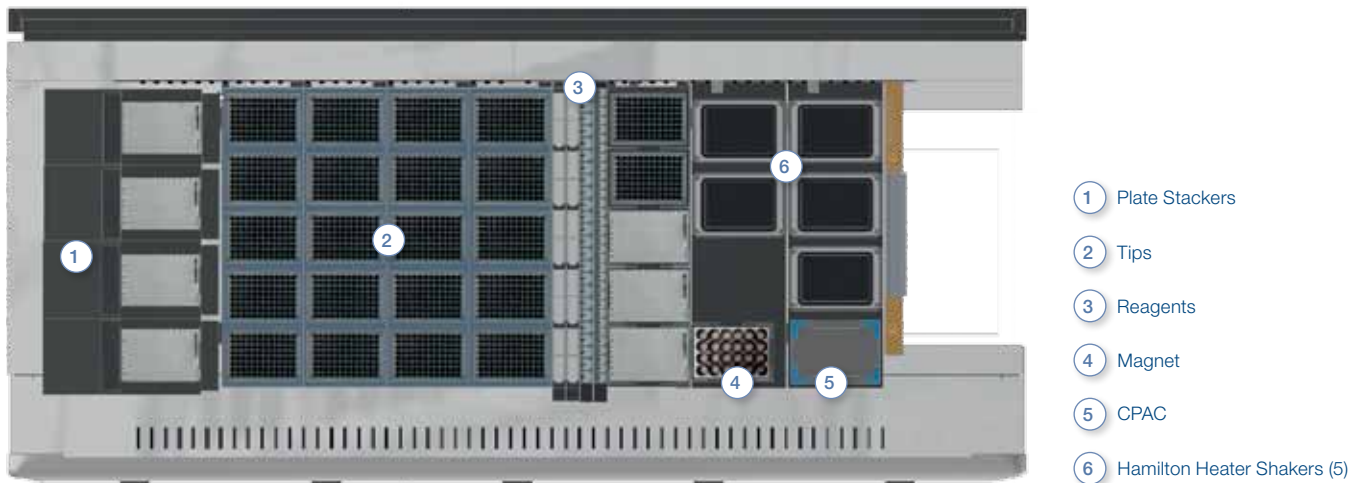
## Description and Workflow

This protocol converts total RNA into a library of template molecules of known strand origin and suitable for subsequent cluster generation and DNA sequencing.



\* Incubations are performed on Hamilton Heater Shakers (HHS) set at different temperatures. PCR amplification is performed off-line. Optional on-line Thermal Cycling with ODTC. \*\* Library Validation is performed off-line on 3<sup>rd</sup> party devices.

## Deck Layout



## Recommended Platforms

Platform	Pipetting Channels	96 MPH	Batch size	ODTC *
STARlet	8	No	48 samples	No
STAR	8	No/Yes	96 samples	Yes

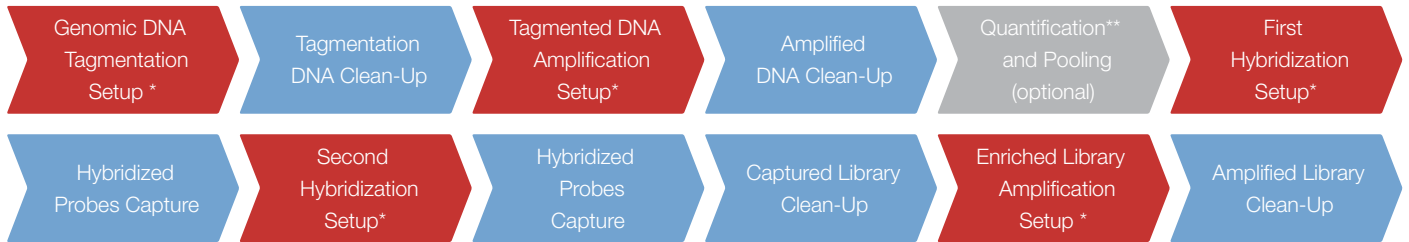
\* On Deck Thermal Cycler



# Illumina Nextera Rapid Capture Exome DNA

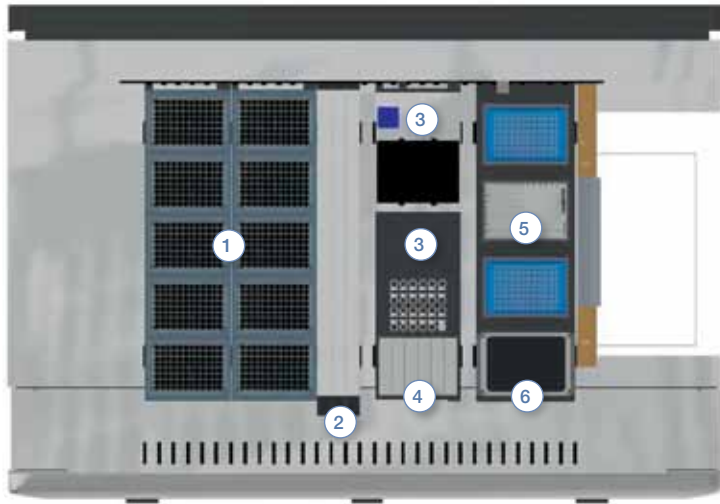
## Description and Workflow

The automated Nextera Rapid Capture protocol is intended to be completed over 2 days by performing library preparation, first hybridization, first capture and second hybridization setup on day 1, final capture, PCR enrichment and final clean-up on day 2.



\* Off line incubation on Thermal Cycler. Optional on line incubation with ODTC  
\*\* Quantification is performed off line on 3rd party devices

## Deck Layout



- 1 Tips
- 2 DNA Samples
- 3 4°C Position
- 4 Reagents
- 5 Magnet
- 6 Hamilton Heater Shaker

By using Hamilton technology and the expertise of their support team NGS libraries can be prepared in a reliable and reproducible way. This allows us to be more committed on patient's health.

Dr. Maria Iacone, Ph.D  
Lab Genetica Molecolare - USSD LGM, AO Papa Giovanni XXIII

## Recommended Platforms

Platform	Pipetting Channels	96 MPH	Unattended batch size	ODTC *
STARlet	4	No	24 samples	No
STARlet	8	No	48 samples	No
STAR	8	Yes	96 samples	Yes

\* On Deck Thermal Cycler



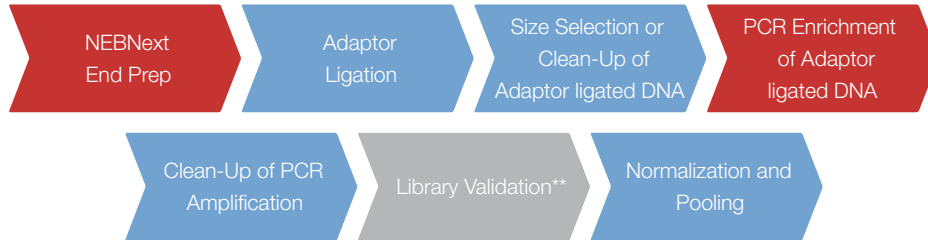




# NEBNext Ultra DNA Library Prep for Illumina

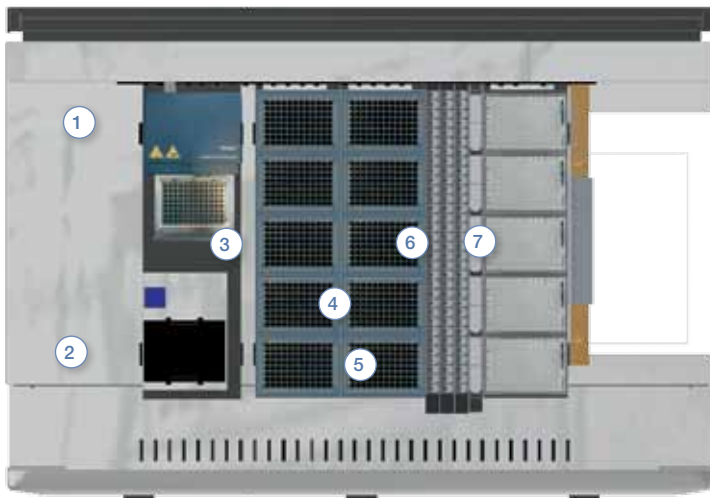
## Description and Workflow

The NEBNext Ultra DNA library preparation kit for Illumina contains enzymes and buffers to convert a small amount of DNA input into indexed libraries for next generation sequencing on the Illumina platforms.



\*\* Library Validation is performed off-line on 3<sup>rd</sup> party devices.

## Deck Layout



- ① On Deck Thermal Cycler (ODTC)
- ② Cooling module for NEB reagents
- ③ Disposable Tips
- ④ Microtubes, fragmented DNA
- ⑤ Reagents
- ⑥ Plates, magnet and primers
- ⑦ Liquid waste

## Recommended Platforms

Platform	Pipetting Channels	96 MPH	Batch size	ODTC *
STARlet	8	No	48 samples	Yes

\* On Deck Thermal Cycler



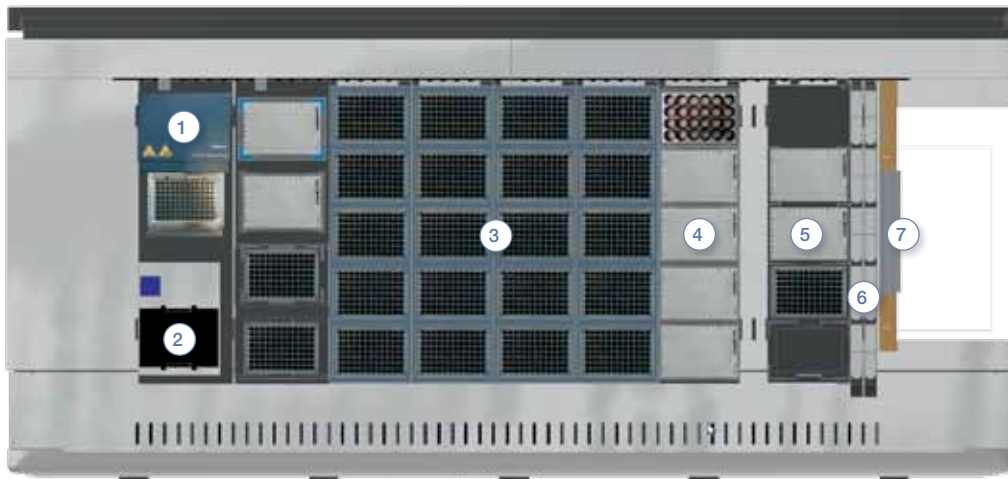
# NEBNext rRNA Depletion Kit (human/mouse/rat)

## Description and Workflow

The NEBNext rRNA Depletion Kit (Human/Mouse/Rat) depletes both cytoplasmic (5S rRNA, 5.8S rRNA, 18S rRNA and 28S rRNA) and mitochondrial ribosomal RNA (12S rRNA and 16S rRNA) from human total RNA preparations. This kit is suitable for both intact and degraded RNA (e.g. FFPE RNA). The resulting rRNA-depleted RNA is suitable for RNA-Seq, random-primed cDNA synthesis, or other downstream RNA analysis applications.



## Deck Layout



- ① On Deck Thermal Cycler (ODTC)
- ② Cooling module for NEB reagents
- ③ Disposable Tips
- ④ Plate stackers, PCR lids
- ⑤ Plates, magnets and primers
- ⑥ Reagents
- ⑦ Liquid waste

## Recommended Platforms

Platform	Pipetting Channels	96 MPH	Batch size	ODTC *
STAR	8	No	48 samples	Yes

\* On Deck Thermal Cycler

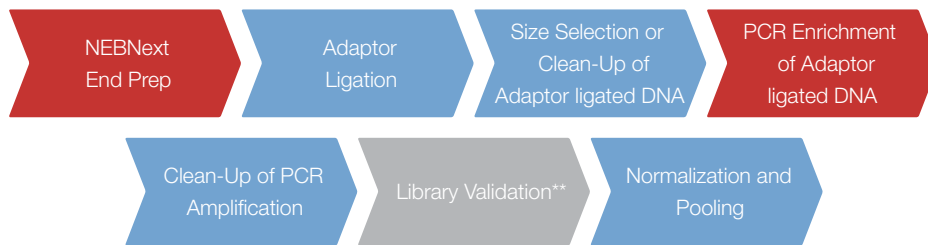




# NEBNext Fast DNA Library Prep for Ion Torrent

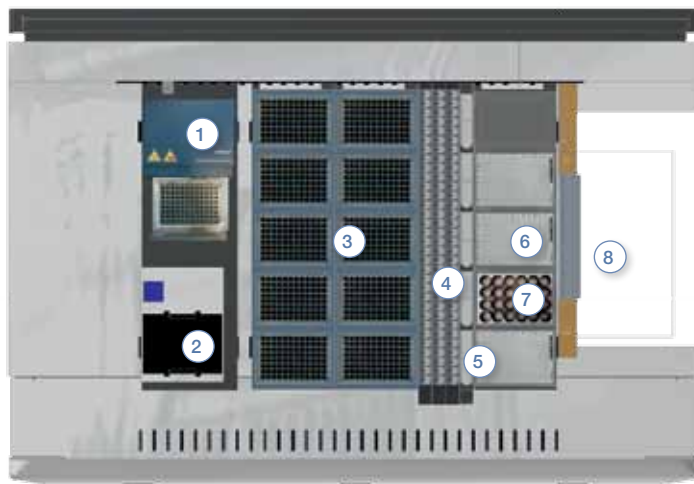
## Description and Workflow

The NEBNext Ultra DNA library preparation kit for Ion Torrent contains enzymes and buffers in convenient master mix formulations that are suited for samples preparation for next generation sequencing on the Ion Torrent sequencer (Life Technologies, Inc.).



\*\* Library Validation is performed off-line on 3<sup>rd</sup> party devices.

## Deck Layout



- ① On Deck Thermal Cycler (ODTC)
- ② Cooling module for NEB reagents
- ③ Disposable Tips
- ④ Microtubes, fragmented DNA
- ⑤ Reagents
- ⑥ Plates and primers
- ⑦ Magnet
- ⑧ Liquid waste

## Recommended Platforms

Platform	Pipetting Channels	96 MPH	Batch size	ODTC *
STARlet	8	No	48 samples	Yes

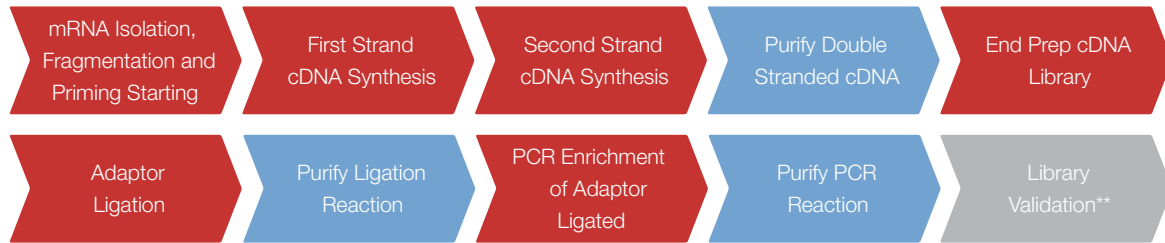
\* On Deck Thermal Cycler



# NEBNext Ultra Directional RNA Libr Prep for Illumina

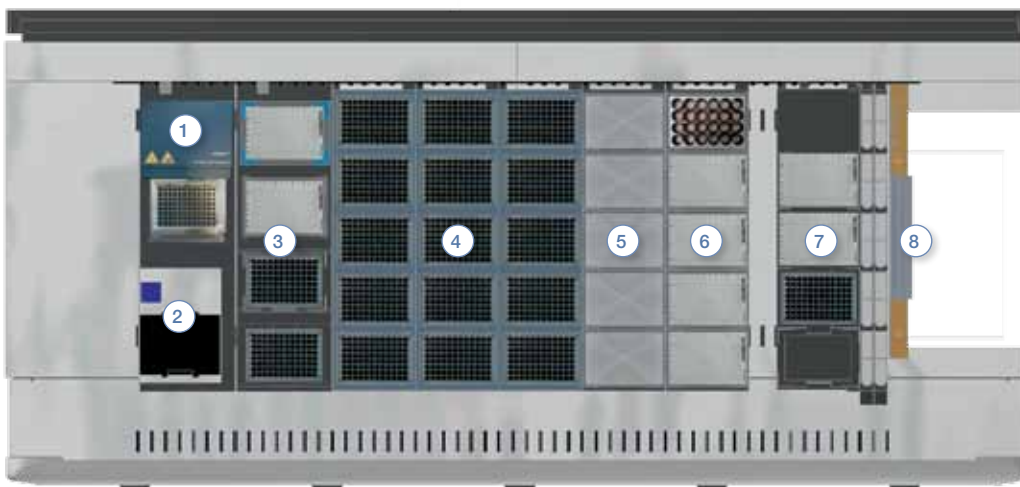
## Description and Workflow

The NEBNext Ultra RNA Directional Library Prep Kit for Illumina contains enzymes and buffers that are ideally suited for cDNA library preparation for next-generation sequencing.



\*\* Library Validation is performed off-line on 3<sup>rd</sup> party devices.

## Deck Layout



- ① On Deck Thermal Cycler (ODTC)
- ② Cooling module for NEB reagents
- ③ Heated Shaker, cooling position
- ④ Disposable Tips
- ⑤ Reagents
- ⑥ Plates, magnet and primers
- ⑦ Plate stackers, PCR lids
- ⑧ Liquid waste

## Recommended Platforms

Platform	Pipetting Channels	96 MPH	Batch size	ODTC *
STAR	8	No	48 samples	Yes
STAR	8	Yes	96 samples	Yes

\* On Deck Thermal Cycler



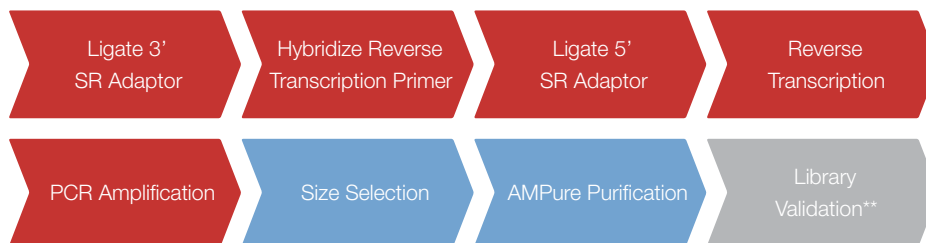




# NEBNext Small RNA Library Prep Set for Illumina

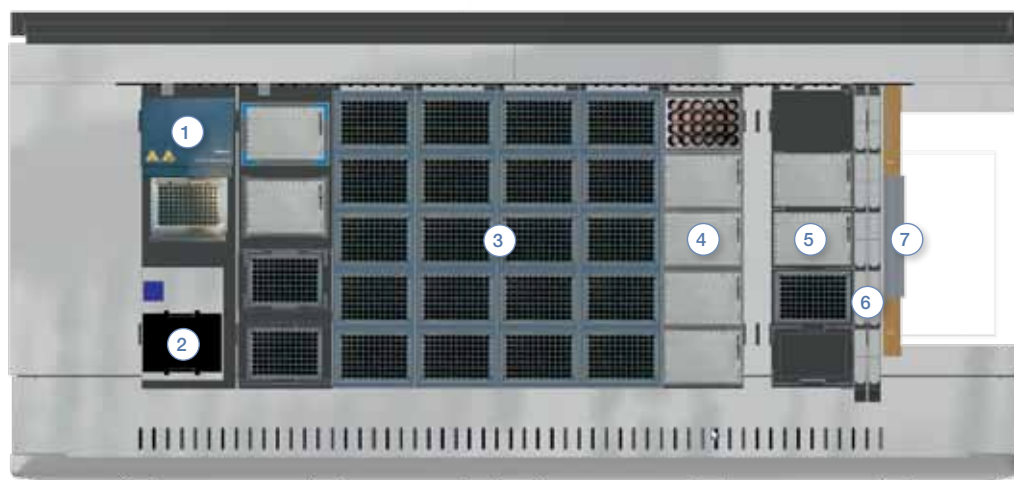
## Description and Workflow

The NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible) contains adaptors, primers, enzymes and buffers that are ideally suited to convert small RNA transcripts into barcoded cDNA libraries for next-generation sequencing on the Illumina platform (Illumina, Inc.).



\*\* Library Validation is performed off-line on 3<sup>rd</sup> party devices.

## Deck Layout



- 1 On Deck Thermal Cycler (ODTC)
- 2 Cooling module for NEB reagents
- 3 Disposable Tips
- 4 Plate stackers, PCR lids
- 5 Plates, magnets and primers
- 6 Reagents
- 7 Liquid waste

## Recommended Platforms

Platform	Pipetting Channels	96 MPH	Batch size	ODTC *
STAR	8	No	48 samples	Yes

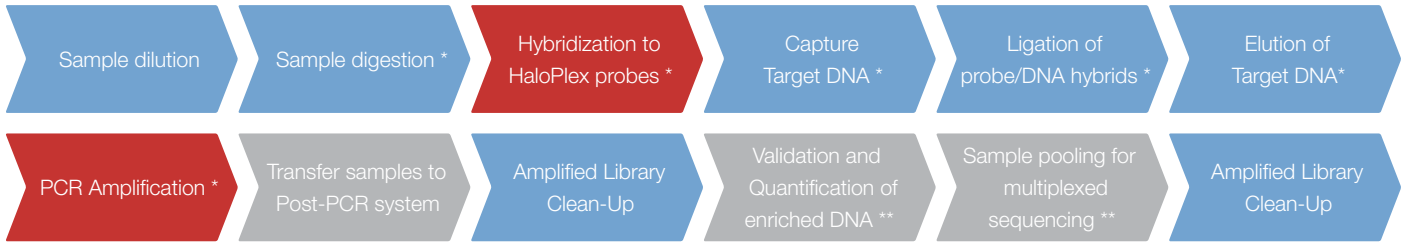
\* On Deck Thermal Cycler



# HaloPlex Target Enrichment System

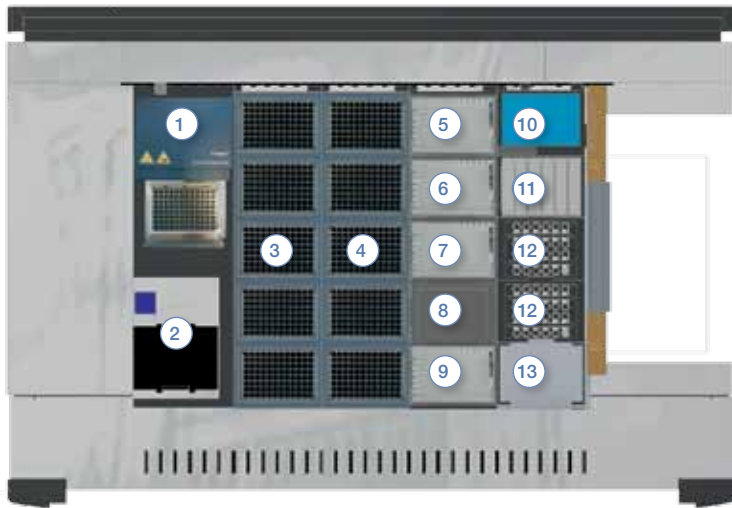
## Description and Workflow

The optimized automation protocol for preparing target enriched sequencing library samples for Illumina sequencing platforms consists of a Pre- and Post-PCR system and is intended to complete a maximum of 24 samples per run.



\* Mastermix set up and shaking steps offline, automation optional  
\*\* Quantification is performed off line on 3rd party devices, as well as sample pooling

## Deck Layout



- ① On Deck Thermal Cycler
- ② MultiFlex Cooling module
- ③ CO-RE Tips, 50 µl
- ④ CO-RE Tips, 300 µl
- ⑤ Sample/Dilution plate
- ⑥ Reaction plate
- ⑦ Primer Cassette plate
- ⑧ Magnet position
- ⑨ Plate stack
- ⑩ Liquid Waste
- ⑪ Reagent Containers  
- AMPure beads  
- Ethanol  
- Elution Buffer
- ⑫ Tube positions
- ⑬ Lid stack

## Recommended Platforms

Platform	Pipetting Channels	96 MPH	Batch size	ODTC **
STARlet ML *	4	No	12 samples	No
STARlet ML *	8	No	24 samples ***	Yes
STAR AL *	8	No	48 samples ***	Yes

\* ML: Manual Load, AL: Autoload

\*\* On Deck Thermal Cycler

\*\*\* Optional tip stacks or tip reloading necessary



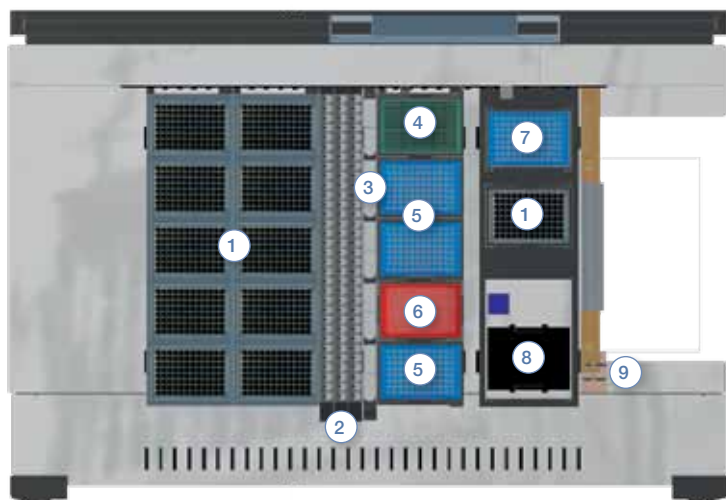
# Ion AmpliSeq Library Prep for Ion Torrent

## Description and Workflow

The automated AmpliSeq Library Preparation protocol offers a fully automated walk-away solution. The system performs all the steps of the protocol started from partial primer digest, barcode adapter ligation through final clean-up steps. Dynamic assignment of barcode adapters to samples is possible and managed by worklists. All steps are done on-deck including thermal incubations. Subsequent qPCR protocols and library pooling methods are available.



## Deck Layout



- 1 Tips 50  $\mu$ l, 300  $\mu$ l
- 2 3x Sample Carrier 32 for 1,5 ml Eppendorf Tubes
- 3 5x 60 ml troughs Ethanol 70%, water, liquid waste, beads
- 4 Barcode Adapter Plate
- 5 Pipetting Positions for PCR plates
- 6 Magnet
- 7 CPAC (Inheco) (4-110°C)
- 8 Cooling Module (cooling of reagent tubes)
- 9 CO-RE Gripper

## Recommended Platforms

Platform	Pipetting Channels	96 MPH	Batch size	ODTC *
STARlet	4	No	24 samples	No
STARlet	8	No	48 samples **	No
STAR	8	Yes	96 samples **	Yes

\* On Deck Thermal Cycler

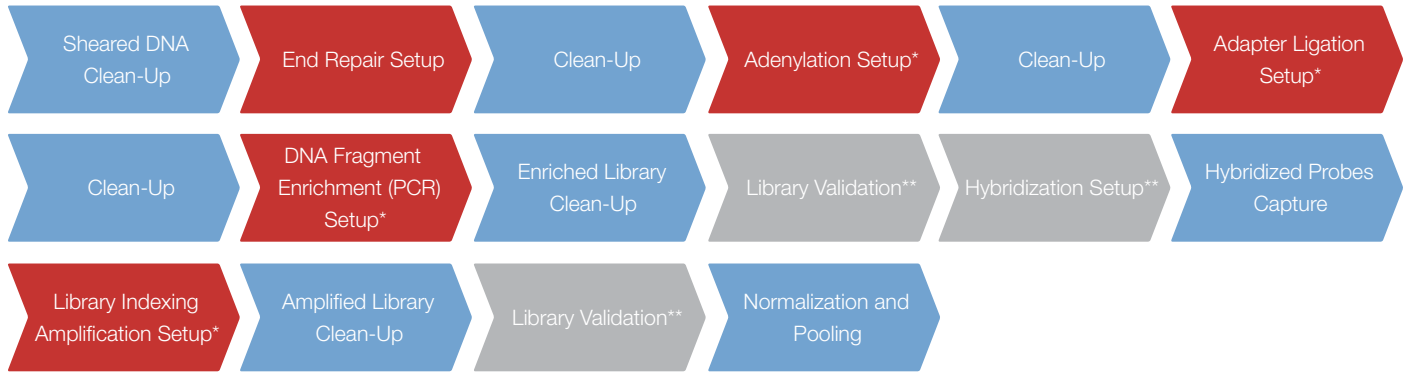
\*\* Actual throughput evaluated on case by case basis



# Agilent SureSelect XT

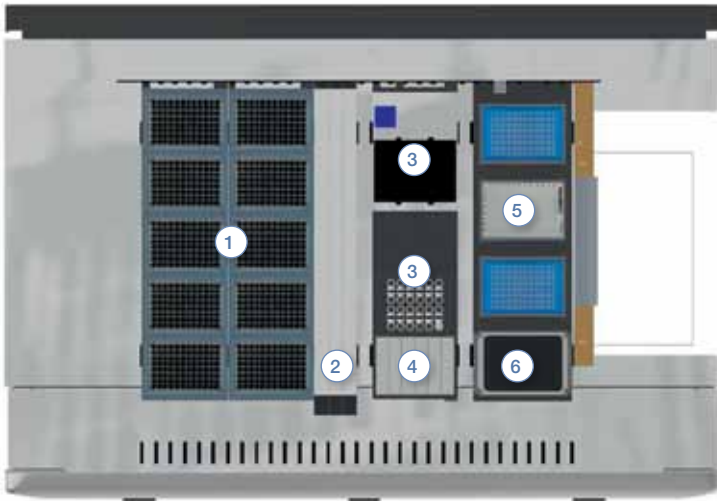
## Description and Workflow

The automated workflow completes all Sure Select protocol steps, preparing libraries from sheared DNA, and includes post hybridization probes capture, library indexing and pooling.



\* Incubations are performed on Hamilton Heater Shakers and Inheco CPACs at different temperature. PCR amplification is performed off line. Optional on deck Thermal Cycling with ODC option.  
 \*\* Library Validation and Hybridization Setup are performed off line.

## Deck Layout



- ① Tips
- ② DNA samples
- ③ 4° C Cooled Positions
- ④ Reagents
- ⑤ Magnet
- ⑥ Hamilton Heater Shaker

## Recommended Platforms

Platform	Pipetting Channels	96 MPH	Unattended batch size	ODTC *
STARlet	4	No	24 samples	No
STARlet	8	No	48 samples	No
STAR	8	No/Yes	96 samples	Yes

\* On Deck Thermal Cycler

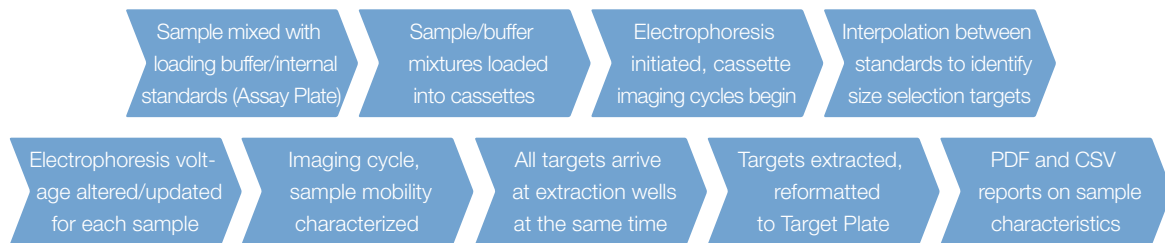




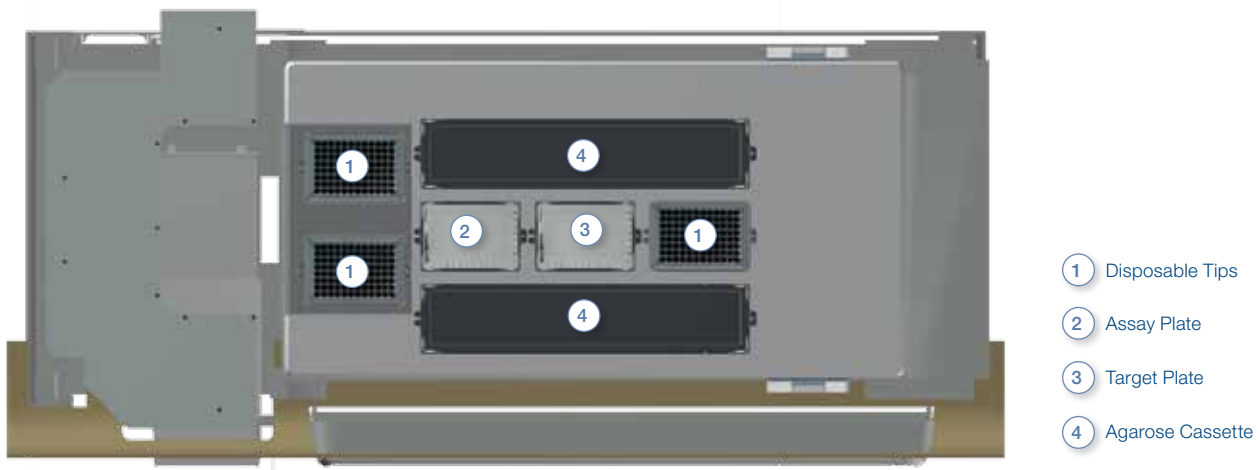
# Coastal Genomics Ranger Technology - Size Selection and Fragment Analysis

## Description and Workflow

Ranger® Technology offers the complete automation of agarose gel loading, electrophoretic analysis and recovery of targeted DNA fragments. Users can consolidate analytical and size selection processes into a single run that can accommodate 1 to 96 samples. Groups interested in analytics without size selection can process up to 384 samples in a single run as well.



## Deck Layout

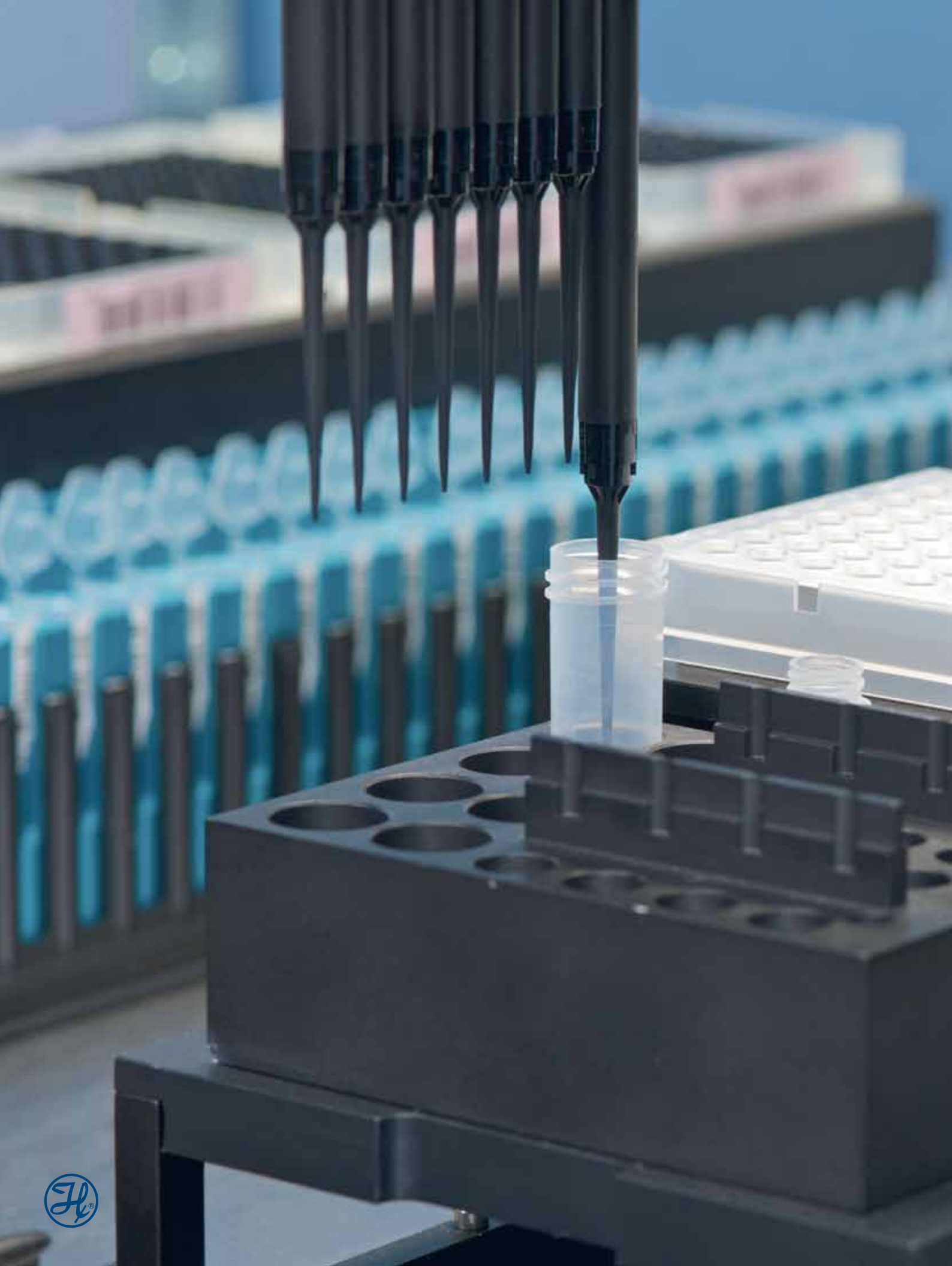


## Recommended Platforms

Platform	Pipetting Channels	96 MPH	Batch size	ODTC *
NIMBUS	n.a.	No	96 samples	No

\* On Deck Thermal Cycler





# About Hamilton Company

Hamilton Company is a global enterprise with headquarters in Reno, Nevada; Franklin, Massachusetts; and Bonaduz, Switzerland and subsidiary offices throughout the world.

We are an industry leader in the design and manufacture of liquid handling, process analytics, robotics and automated storage solutions. For more than 60 years, Hamilton has been satisfying customer needs by combining quality materials with skilled workmanship to ensure the highest level of performance. Hamilton's lifelong commitment to precision and quality has earned us global ISO 9001 Certification.



Founded on the technology of analytical Microliter™ and Gastight® syringes, Hamilton has a broad offering of laboratory products including manual and semi-automated precision fluid measuring instruments, chromatography products, process sensors, laboratory electrodes, pipettes and more. Top innovations from these lines include Arc™ pH, DO and Conductivity Intelligent Sensors, the BioLevigator™ 3D Cell Culture System, Microlab® 600 Diluters/Dispensers and the Microlab® 300 Guided Pipetting System.

A pioneer in liquid handling equipment and laboratory automation technology, Hamilton Robotics is known for advancing life science and biotechnology industries through reliability, performance and flexibility. Hamilton is the industry leader in design and manufacturing with patented technologies such as Compression-induced O-Ring Expansion (CO-RE®), Total Aspiration and Dispensing Monitoring (TADM) and Anti-Droplet Control (ADC™). Hamilton's platforms include Hamilton VANTAGE Liquid Handling System™, its newest vertically-integrated liquid handler, Microlab® STAR, Hamilton's highest selling automated pipetting platform, and Microlab NIMBUS®, the first in its class of compact, high-speed, personalized pipetting workstations.



Hamilton Storage Technologies offers comprehensive ultra-low temperature automated sample management systems for microtube and microplate storage. Hamilton's line of biobanking and compound storage solutions, as well as consumables, are designed for a broad array of life science processes. Products include BIOS™, SAM™ and ASM™, designed for sample integrity, flexibility and reliability.

Hamilton Company is focused on blending invention and accuracy to deliver customers unparalleled products.



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Web: [www.hamiltonrobotics.com](http://www.hamiltonrobotics.com)

USA: 800-648-5950

Email: [infoservice@hamiltonrobotics.com](mailto:infoservice@hamiltonrobotics.com)

**United States**

Tel: +1-775-858-3000

**United Kingdom & Ireland**

Tel: +44 (0)121-717-0199

**Brazil**

Tel: +55 (11) 126-50562

**China**

Tel: +86-21-6164-6567

**France**

Tel: +33 (01) 69751616

**Italy**

Tel: +39-39-689-33-93

**Denmark, Norway,  
Sweden, Finland**

Tel: +45-70-26-4499

**Germany, Switzerland,  
Austria, Benelux**

Tel: +49 (089) 552649-0

To find a subsidiary or distributor in your area, please visit [hamiltonrobotics.com/contacts](http://hamiltonrobotics.com/contacts).