How Vendor-Qualified Automated Methods Accelerate NGS Workflows

From Bench to Sequencing in Days

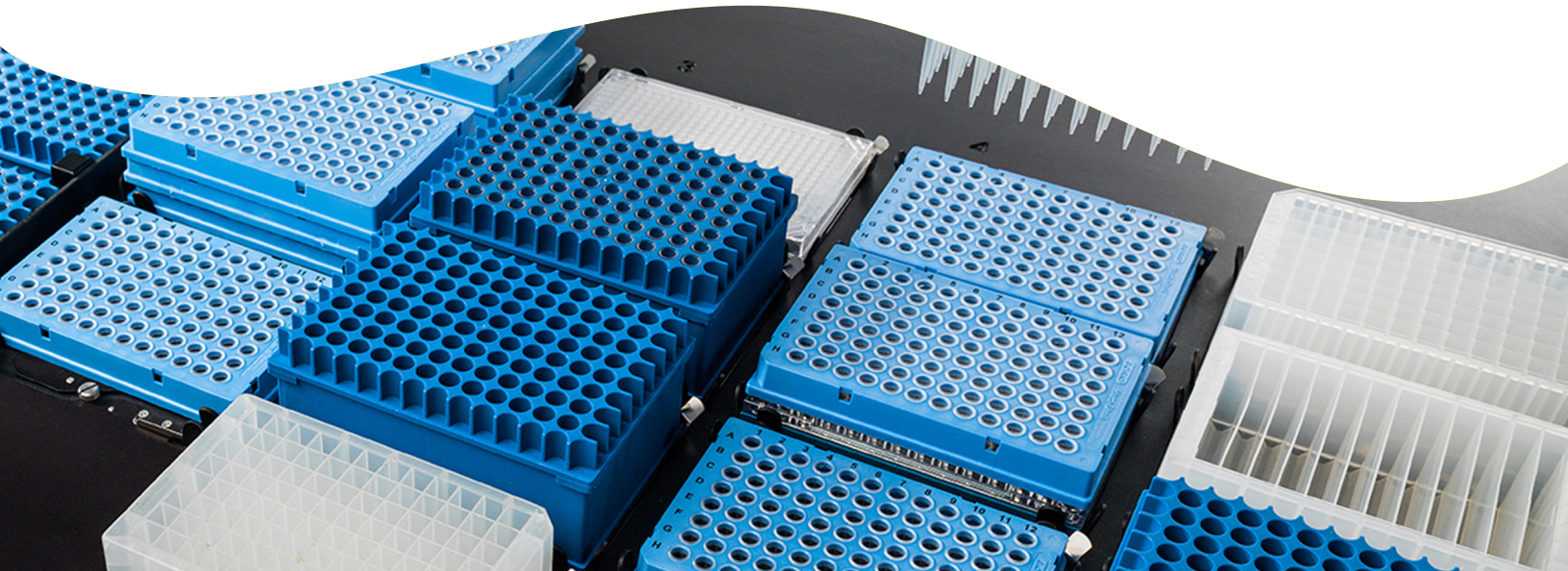
Key features

- Protocol Developed, Software Coded Automated Methods
- Fully Vendor-Qualified NGS Libraries
- Understanding the Science of NGS

Introduction

With the cost to sequence genomes declining dramatically, scientists and researchers are applying next generation sequencing (NGS) technology to new discoveries and clinical research applications at a rapidly expanding rate. These applications are driving the need for sample preparation workflows that prepare the DNA/RNA libraries prior to sequencing. This growing appetite for new kits plus the demand for higher-throughput solutions often mean that start-up to sequencing can take months. For the laboratory manager and user, the challenge is not installing and starting to use the automated sample preparation system, it is the difficult and time-consuming steps to get the automated methods to create the proper libraries that are required for sequencing and the generation of high-quality genomic data.

The development of vendor-qualified, automated library protocols offers a solution to this growing problem. This scientific brief examines how vendor-qualified automated library protocols are shrinking the time laboratories spend to start sequencing from months to days. It describes how installing automation with a vendor-qualified method eliminates much of the on-site application development, field service, and support work that are required by a custom or unqualified automated method. It also reviews the three steps of library readiness – software-protocol, water- and chemistry-tested, and vendor-qualified – and describes how each one can impact start-up times.



Some automation suppliers are beginning to provide vendor-qualified automated library protocols as part of their NGS sample preparation offerings. For example, one automation company, Revvity, pioneered this approach and has developed over 130 of these vendor-qualified automated library protocols with additional ones continuously in development.

The Three Levels of Automated Library Preparation Readiness

When a laboratory is considering the decision and deployment of automation for an NGS library preparation kit, it is important to understand the three levels of automation solution readiness prior to making a decision.

Protocol Developed, Software Coded

The software program installed in the automation system is the low-level coding that controls all workflow steps and methods. Some laboratories will buy automation hardware where the software protocols are already written for the DNA/RNA kits, but the NGS kit and chemistries are not yet created or tested.

In this mostly do-it-yourself approach, the automation hardware will be installed by the vendor, but the laboratory user must test and refine the software protocol, test the chemistry, update the protocol, qualify the application, sequence the samples, and verify the results. NGS sample preparation protocols are complex and have many different steps such as shaking, incubation, mixing, and thermocycling. Ensuring the chemistry works to vendor specifications can be very time consuming. The testing and qualification processes can require months of trial-and-error work, particularly if the automation supplier's field service technicians do not understand the science behind NGS kits, have limited NGS expertise, or do not provide testing support. Sample preparation and sequencing are expensive processes. In this scenario the customer must incur these costs and time if they decide to purchase an automation system with only the protocol written but must perform the testing and qualification work themselves.

It is not just the software protocol coding, it is ensuring that what you have coded has dialed in the chemistry properly to ensure the libraries meet vendor specifications for sequencing. You can quickly code the automation's ability to move plates around and make liquid transfers but ensuring that the chemistry works to vendor specifications can be very time consuming.

Water & Chemistry Tested with Quality-Control (QC) Results

At this level of readiness, the automation vendor has developed the software protocol and then must perform water and chemistry testing and QC analysis. Water testing is performed to make sure the application's liquid transfer scheme performs as expected. The vendor should run at least 7 columns of samples to ensure the automation achieves the correct calculations for master mix and tip logic.

The vendor also conducts chemistry testing to confirm the system's pipetting scheme is accurate and that there are no complications between plates and the deck layout. Finally, QC is performed to ensure the NGS libraries are of the correct yield and size. This is normally done by testing on instruments such as ThermoFisher Qubit, Revvity LabChip GX Touch or Agilent TapeStation instruments. The vendor will begin the testing with a small sample set (3 columns), conduct quality control, and if correct, continue with full-plate chemistry testing. Even with this more thorough testing and QC, no sequencing has been performed on the libraries. Therefore, after automation system installation, the customer must still prepare and sequence the DNA/RNA libraries at their site to confirm it meets their quality and compliance needs. It is not uncommon that sequencing may show problems that the QC did not, such as duplication rate, coverage depth and uniformity, or GC bias. If this happens, the automated protocol will need updates. Therefore, even with this additional vendor chemistry and QC testing, the customer may still spend weeks to months tweaking and further developing the protocol, chemistry, and library creation to get the correct sequencing results.

Fully Vendor-Qualified NGS Libraries

After performing water and chemistry testing and QC analysis in their own applications development laboratories, some automation vendors will send their final prepared DNA/RNA libraries to the NGS kit supplier who sequences the libraries and performs analysis to ensure library quality and all metrics meet their highest compliance and standards. Once acceptable data is produced, the sequencing supplier will approve the application as a vendor-qualified automated library.

At this point, the automation equipment and the automated library preparation kits are ready to be set up at the customer's site. This level of readiness assures the customer the quickest time from installation to sequencing real samples. It gives the customer confidence in the sequenced data since the kit vendor has already qualified the assay performance to ensure that the automated results are equivalent to their own prescribed manual performance and results.

In addition, to ensure customers have confidence in their automation workstations futures, suppliers such as Revvity continually work with kit vendors to update their automation methods if the kit vendor releases a new or updated version.

The Benefits of Vendor-Qualified NGS Library Preparation Assays

The beauty of the vendor-qualified automated library workflow is its rapid time from set-up to sequencing and assurance of quality data. The automation vendor already has invested in the protocol software development, water and chemistry testing, and QC analysis. Plus, the kit vendor has run the library samples through sequencing to ensure the automation data is equivalent to their manually generated data. All of this development and qualification work is performed before the automation equipment is installed at the customer's laboratory.

The automation vendor's field applications specialist will install and test the equipment and protocol and provide user training on the application. They will ensure the automation is performing as expected and the user is fully able to operate the system. Finally, libraries will be created and analyzed for quality control before being sequenced to show quality data. Overall, after the equipment is installed, the customer can be sequencing libraries in as few as 5 days (see figure 1).

If the laboratory's kit requires some customization, such as modification of PCR cycles, the software interface on a vendor-qualified solution should enable simple user configurations while still meeting kit vendor requirements. These adjustments should include user variables such as incubation and fragmentation times and PCR cycles.

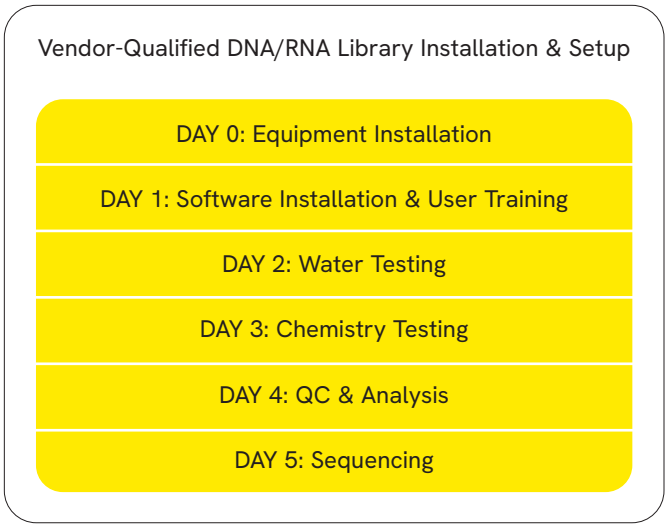


Figure 1: Once the equipment is installed, the setup of a vendor-qualified DNA/RNA library is the fastest path from installation to sequencing and typically requires about 5 days. (Source: Revvity Field Application Services)

The installation and setup of an automated vendor-qualified DNA/RNA library represent the fastest path from installation through QC to sequencing. For the laboratory manager, it means:

- Faster time to revenue operations and asset utilization
- More time spent on production, less on development
- No employee programming knowledge required
- Greater flexibility to react to market conditions and opportunities
- Automation that is future proof
- Employees engaged in higher-value work
- Rapid automation of emerging methodologies

Now, laboratory employees can spend more time on sequencing and minimal time on library preparation, testing, and qualification.

For the laboratory user, a vendor-qualified automated method also offers attractive benefits, such as:

- Fastest automation start-up
- Quickest time to deploy additional libraries
- More efficient use of samples
- Access to automated protocols for future kits

Case Study: The Trials of Deploying a Custom Automated NGS Method

An emerging biotech company required an automated, high-throughput NGS library preparation system with an integrated, on-deck thermocycler to run a kit they currently performed manually. The customer purchased automation with the capability to process two, 96-plate assays. While the vendor promised they would install the system and get the assay up and running quickly, initial operation proved otherwise. The first sample preparation runs did not deliver the expected throughput, and the DNA library did not provide acceptable data due to a faulty sample preparation process. The laboratory conducted a number of trial-and-error optimization routines over 18 to 24 months until it generated valid sequencing data. Two years after the installation, the laboratory only had one method running of the several it wanted to apply, and it was operating with only a single 96-well plate workflow.

While this is an extreme case, it highlights the difficulty of deploying an unqualified custom automated method. On average, it can require about 6 to 9 months to get an automated custom sample preparation method installed and sequencing acceptable results.

Case Study: 5 Days to Sequenced Data at a Large Pharmaceutical Company

A large pharmaceutical company needed rapid start-up of an NGS sample preparation automated method. Its sequencing laboratory purchased a high-throughput automation system and a vendor-qualified DNA library preparation protocol. The system included the operating software, library preparation protocols, and all the documentation and workbooks needed to install and support the solution. The supplier's service team set up the automation equipment.

Day 1: The field application specialist imported the automated protocols and configuration to the automation system. This work included calibrating the liquid handler positions to ensure they performed accurately.

Day 2: The field application specialist trained the laboratory's NGS team on how to use the software and its user interface.

Day 3: Water testing was conducted with the liquid handler filling and running the plates with the volumes specified in the setup guide. The users also ran chemistries on the system to ensure compliance with kit specifications.

Day 4: The customer executed an initial QC protocol to confirm the samples met the specified concentrations and yields and were ready for sequencing.

Day 5: The laboratory started sequencing samples and generated acceptable data.

Understanding the Science of NGS

While many automation providers are experts at programming a liquid handler to mimic a manual workflow, they do not necessarily understand the science of NGS. Their automation product lines are wide, and NGS is not their specialty. When purchasing an automated NGS sample preparation solution, the customer should ensure the vendor has an applications development group and field applications team that are knowledgeable about NGS science and how to automate it.

These valuable resources accelerate the installation and setup of custom automated methods, can quickly overcome the challenges of new chemistries, and can adjust sample preparation methods as vendors develop new kits or versions. They also can train the lab employees on how to apply the chemistries as well as operate the automation.

Conclusion

NGS labs are under considerable pressure to accelerate their times to sequencing, discovery, and revenue. Laboratory managers need lower costs per sample, higher throughput, reduced staff training times, and future-proof solutions to ensure they meet their facility's goals. Laboratory users want simple-to-use automation, fast start up times, easier and more efficient operation, faster changeovers, and better error recovery. NGS labs are under considerable pressure to accelerate their times to sequencing, discovery, and revenue. Laboratory managers need lower costs per sample, higher throughput, reduced staff training times, and future-proof solutions to ensure they meet their facility's goals. Laboratory users want simple-to-use automation, fast

start up times, easier and more efficient operation, faster changeovers, and better error recovery. With library prep solutions already proven with sequencing results, laboratories can make considerable progress in reducing the time to start up new sequencing kits and data generation. As NGS laboratories evaluate new automated sample preparation solutions, focusing on vendors who have vendor-qualified automated library protocols can play a key role in meeting their objectives. With library prep solutions already proven with sequencing results, laboratories can make considerable progress in reducing the time to start up new sequencing kits and data generation. As NGS laboratories evaluate new automated sample preparation solutions, focusing on vendors who have vendor-qualified automated library protocols can play a key role in meeting their objectives.

Today, these automated library protocols cover most NGS kits and save time and effort during new system installation and start up as well as when deploying new automated methods on existing automation systems. In the future, if different NGS automated methods may be required, it is important to engage with a supplier who will have those new automated methods available in a vendor-qualified status.

Buying an NGS sample preparation system should not involve spending weeks or months of development and testing all while keeping your fingers crossed the created libraries will sequence properly. Instead, laboratories should consider vendor-qualified automated solutions that offer the fastest times to sequencing with proven qualified results.

Revvity has pioneered the development of vendor-qualified DNA/RNA libraries and has produced over 130 of these automated library protocols. Additional ones are continuously in development. They support nearly all kit vendors and applications in the NGS sequencing market. To learn more **click here.**

Fast and Easy Library Prep Methods for Your NGS Workflow - DNA Library Preparation Kits

Company	Product	Sciclone® G3 NGSx	Sciclone® G3 NGSx iQ™	Zephyr® G3	BioQule™ NGS System
Revvity	NEXTFLEX® Rapid XP V2 DNA-Seq Kit	X	X	X	X
Revvity	NEXTFLEX® Rapid DNA-Seq Kit 2.0	X	X	X	X
Revvity	NEXTFLEX® Cell Free DNA-Seq Kit 2.0	X	X	X	
Revvity	PG-Seq® Kit	X			
10x Genomics®	Genomic Preparation	X			
ArcherDx®	Universal DNA Reagent Kit v2	X			
CareDx®	AlloSeq Tx			X	
IDT®	xGen DNA Library Prep Kit EZ		X		
Illumina®	Nextera® XT DNA Kit	X	X	X	
Illumina®	TruSeq® DNA PCR-Free Kit	X	X		
Illumina®	TruSeq® Nano DNA Kit	X			
Illumina®	Illumina® DNA Prep	X	X	X	
Illumina®	Illumina® DNA Prep with Enrichment	X	X		
Illumina®	DNA PCR-Free Library Prep Tagmentation	X	X		
NEB®	NEBNext® Ultra™ II DNA Library Prep for Illumina® Instruments	X		X	
NEB®	NEBNext® Ultra™ DNA Kit	X		X	
NEB®	NEBNext® dsDNA Fragmentase™ Kit	X			
NuGen®	Celero DNA-Seq		X		
NuGen®	Ovation® Ultralow DR Multiplex System (1-96)	X		X	
PacBio®	2kb Template Preparation & Sequencing	X	X	X	
PacBio®	10kb Template Preparation & Sequencing	X	X	X	
PacBio®	20kb Template Preparation Using BluePippin™ Size-Selection System	X		X	
PacBio®	Greater than 10kb Template Preparation Using AMPure® PB Beads	X		X	
PacBio®	HiFi SMRTbell® Libraries with SMRTbell Prep Kit 3.0	X	X		
PacBio®	Multiplexed Microbial Library Prep with SMRTbell Express Template Prep Kit 2.0	X	X		
Roche®	DNA HTP Library Preparation Kit	X		X	
Roche®	DNA HyperPrep™ Kit	X	X	X	
Roche®	DNA HyperPlus™ Kit	X	X	X	
Roche®	KAPA ExoPlus Kit	X			
Thermo Fisher®	Ion Torrent™ Ion Express® Fragment Library Prep Kit	X		X	
Thermo Fisher®	Ion Express® Plus gDNA Fragment Library Prep Kit	X		X	
TwinStrand™ Bioscience	TwinStrand Duplex Sequencing™: Pre-PCR		X		
TwinStrand™ Bioscience	TwinStrand Duplex Sequencing™: Post-PCR			X	
Twist®	Library Preparation Kit	X	X		
QIAGEN®	QIAseq® FX DNA Library Kit	X			
QIAGEN®	QIAseq® Targeted DNA v3 Panel	X	X		
IDT®/Swift®	Accel-NGS® 25 Plus DNA Library Kit	X			
IDT®/Swift®	xGen Amplicon Panels		X		

Fast and Easy Library Prep Methods for Your NGS Workflow - RNA Library Preparation Kits

Company	Product	Sciclone® G3 NGSx	Sciclone® G3 NGSx iQ™	Zephyr® G3
Revvity	NEXTFLEX® Rapid Directional RNA-Seq Kit with Poly(A) Beads 2.0	X	X	X
Revvity	NEXTFLEX® Combo-Seq™ mRNA/miRNA Kit	X	X	
10x Genomics®	Chromium™ Single Cell 3' Library Kit v3			X
Agilent®	SureSelect® Strand-Specific RNA Library Prep Kit		X	
Illumina®	RNA Prep with Enrichment (L) Tagmentation	X	X	
Illumina®	TruSeq® RNA Access Kit	X		
Illumina®	Stranded mRNA prep	X	X	
Illumina®	TruSeq® Stranded mRNA (poly-A selection) Kit	X	X	X
Illumina®	TruSeq® Stranded Total RNA™ Kit	X	X	X
Illumina®	Stranded Total RNA Prep, Ligation with Ribo-Zero Plus	X		
Illumina®	TruSeq® RNA Library Prep Kit v2	X	X	X
Illumina®/Epicentre	Ribozero™/Scriptseq™ Stranded RNA Kit	X		
Lexogen®	autoSENSE™ mRNA-Seq Library Prep Kit for Illumina® Sequencing	X		
NEB®	NEBNext® Ultra™ RNA Library Prep Kit for Illumina® Sequencing	X		X
NEB®	NEBNext® Ultra™ RNA II Library Prep Kit for Illumina® Sequencing	X		
NuGen®	Ovation® Human Blood RNA-Seq Library Systems	X		
NuGen®	Ovation® Human FFPE RNA-Seq Multiplex System	X		
NuGen®	Ovation® RNA-Seq System v2	X		
NuGen®	Ovation® Universal RNA-Seq System (strand-specific)	X		
PacBio®	HiFiViral SARS-CoV-2	X		
QIAGEN®	QIASeq® DIRECT SARS-CoV-2	X	X	
QIAGEN®	QIASeq® miRNA	X		
Roche®	RNA HyperPrep™ Kit	X	X	X
Roche®	RNA HyperPrep™Kit with RiboErase™	X		X
Roche®	Stranded RNA-Seq Kit	X		X
Roche®	Stranded RNA-Seq Kit with RiboErase™	X		X
Roche®	Stranded mRNA-Seq Kit	X		X
Roche®	mRNA HyperPrep™ Kit	X		X
Takara formerly Clontech	SMART-Seq® HT Kit			
Thermo Fisher®	Ion Express™ Plus gDNA Fragment Library Prep Kit	X		
Thermo Fisher®	(Ion Torrent™) Ion Total RNA Kit v2	X		
Twist®	cDNA Library Preparation Kit for ssRNA Virus Detection	X	X	

Fast and Easy Library Prep Methods for Your NGS Workflow - Small RNA Library Preparation Kits

Company	Product	Sciclone® G3 NGSx	Sciclone® G3 NGSx iQ™	Zephyr® G3
Revvity	NEXTFLEX® Small RNA-Seq Kit v3	X	X	X
Revvity	NEXTFLEX® Small RNA-Seq Kv3 Automation Kit with UDIs	X	X	X

Fast and Easy Library Prep Methods for Your NGS Workflow - Exome/Target Capture Library Preparation Kits

Company	Product	Sciclone® G3 NGSx	Sciclone® G3 NGSx iQ™	Zephyr® G3
PerkinElmer	NEXTFLEX® Variant Seq™ SARS-CoV-2 V2 Kit	X	X	X
Agilent®	HaloPlex® Kit	X		
Agilent®	HaloPlex® Kit	X		
Agilent®	SureSelect® XT2 Kit	X	X	X
Agilent®	SureSelect® XT Kit	X	X	
Agilent®	SureSelect® XT HS	X	X	
Agilent®	SureSelect® QXT Kit	X		X
Cergentis®	TLA Targeted Locus Amplification Kit	X		
IDT®/Epicentre	xGen Hybridization and Wash Kit		X	
IDT®/Epicentre	xGen Exome Hyb Panel v2	X	X	
Illumina®	COVIDSeq™ Test	X	X	X
Illumina®	Nextera® Rapid Capture Kit	X		X
Illumina®	TruSeq® Rapid Exome Kit	X		
Illumina®	TruSeq® Custom Amplicon v1.5 Kit			X
Illumina®	TruSeq® Custom Amplicon Low Input Kit	X		X
Illumina®	TruSeq® Exome Kit	X		
Illumina®	TruSeq® Focus Panel	X		
Illumina®	TruSeq® Cancer HotSpot Panel v2	X		
Illumina®	TruSeq® Comprehensive Panel v3	X		
Illumina®	TruSight® Cancer Kit	X		
Illumina®	TruSight® Enrichment Kit			
Illumina®	TruSight® Rapid Capture Kit	X		
Illumina®	TruSight® Tumor Kit			
Illumina®	TruSight® Tumor 15 Kit			
NEB®	NEBNext® Direct® Cancer HotSpot Panel	X		
NuGen®	Ovation® Target Enrichment System	X		
Roche®	KAPA HyperCap v3	X	X	
Roche®	SeqCap® EZ Kit	X		
Roche®	SeqCap® EZ HyperPrep® Kit	X		
Thermo Fisher®	Ion TargetSeq™ Exome Kit			
Thermo Fisher®	Ion Ampliseq® Panels			
Twist®	Human Core Exome Kit	X	X	
Twist®	Twist® Custom Panels	X	X	
Twist®	Fast Hybridization Target Enrichment	X	X	

Fast and Easy Library Prep Methods for Your NGS Workflow - Metagenomics Library Preparation Kits

Company	Product	Sciclone® G3 NGSx	Sciclone® G3 NGSx iQ™	Zephyr® G3
Revvity	NEXTFLEX® Rapid XP DNA-Seq Kit v2	X	X	X
Revvity	NEXTFLEX® 16S V1-V3 Amplicon-Seq Kit	X		
Revvity	NEXTFLEX® 16S V4 Amplicon-Seq Kit 2.0	X		
Revvity	NEXTFLEX® 18S ITS Amplicon-Seq Kit	X		X
Revvity	NEXTFLEX® Rapid DNA-Seq Kit 2.0	X		
Revvity	NEXTFLEX® Rapid DNA-Seq Kit	X		
NuGen®	Ovation® Ultralow Methyl-Seq Library System	X		

Fast and Easy Library Prep Methods for Your NGS Workflow - Supplementary Applications & Protocols

Company	Product	Sciclone® G3 NGSx	Sciclone® G3 NGSx iQ™	Zephyr® G3
PerkinElmer	NEXTFLEX® Poly(A) Bead Selection	X		X
PerkinElmer	Normalization & Pooling Protocol	X		X
Beckman Coulter®	SPRI® Purification Protocol	X		X
Beckman Coulter®	SPRI® Size Selection Protocol	X		X
NuGen®	Encore® Biotin Module	X		
NuGen®	Ovation® FFPE WTA System	X		
NuGen®	Ovation® Pico WTA System v2	X		
Thermo Fisher®	PicoGreen® Quantitation	X		X
Thermo Fisher®	Qubit® Quantitation			X
Thermo Fisher®	Quant-iT® Quantitation			X
Roche®	SYBR® FAST qPCR Kit	X		
Roche®	Library Quant Kit	X		X
Roche®	hgDNA Quantification and QC Protocol	X		X

Revvity
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com

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