Automated Solutions for AmpliSeq™ for Illumina Sequencing Panels

Automated methods from trusted partners provide consistent, reproducible library prep.

Introduction

Consistent, reproducible library preparation is a key factor to ensuring high-quality data in next-generation sequencing (NGS) applications. Manual library prep requires multiple pipetting steps, each presenting an opportunity for user-introduced error. Because of this, researchers may consider automating the library prep phase of the NGS workflow. However, evaluating and adopting commercial automation solutions can be daunting and time-consuming. Illumina facilitates kit automation by partnering with leading automation vendors in liquid handling, fragmentation, and size selection. The resulting solutions significantly decrease the time and expense of method development and optimization.

This application note describes available automated solutions for the AmpliSeq for Illumina targeted resequencing solution.

AmpliSeq for Illumina

AmpliSeq for Illumina offers fast, accurate targeted resequencing that enables researchers to go from DNA or RNA to variant calls in 2.5 days. The supported solution includes curated panel content, a PCR-based library preparation assay, proven Illumina next-generation sequencing (NGS) technology, and user-friendly data analysis (Figure 1). Delivered results provide the high on-target and uniform coverage needed to detect low-frequency variants with consistency and reliability.

Leveraging high-performance AmpliSeq chemistry, researchers can use the AmpliSeq for Illumina assay to focus their studies on specific genes, regions, or variants of interest with high accuracy. Users can take advantage of expertly selected content in ready-to-use panels or custom, made-to-order panels to meet specific needs.

Made-to-order options include custom DNA or RNA panels to target unique areas of interest, preconfigured community panels, and on-demand gene panels for human disease research. The PCR-based library preparation assay uses oligonucleotides to amplify regions of interest to investigate single nucleotide variants (SNVs), insert/deletions (indels), copy number variations (CNVs), gene fusions, and differential gene expression. AmpliSeq chemistry can multiplex 12 to > 24,000 amplicons, enabling simultaneous capture of multiple targets in a single reaction. AmpliSeq for Illumina works with RNA and DNA samples, requiring as little as 1 ng of high-quality DNA or RNA and the flexibility to accommodate formalin-fixed, paraffin-embedded (FFPE) samples, such as preserved tumor tissue.

The AmpliSeq for Illumina solution is optimized for use with Illumina sequencing by synthesis (SBS) chemistry. Prepared libraries can be sequenced on any Illumina sequencing system, including the iSeq™ 100, MiniSeq™, MiSeq™, and NextSeq™ Sequencing Systems.

Automated library prep

The AmpliSeq workflow requires multiple pipetting steps performed by trained lab technicians. Such routine and repetitive processes are highly amenable to laboratory automation solutions. It should be noted that automating the library prep process does not significantly decrease overall processing time as this is typically dictated by incubations and PCR steps. However, automating library prep does allow researchers to spend their time more productively and offers many advantages, including:

- low variability due to reproducible handling of samples
- reduced risk of human error
- less hands-on time

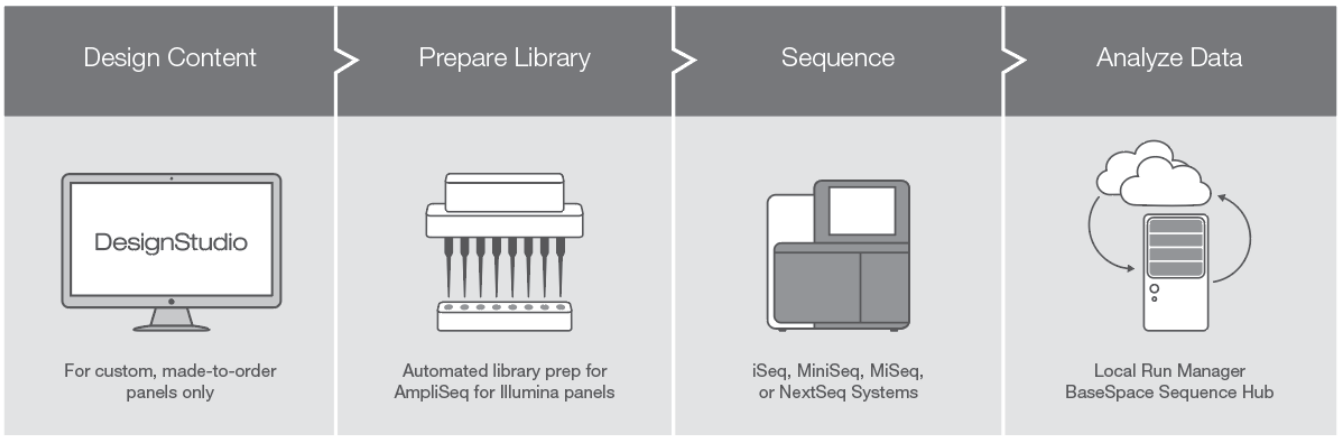

Figure 1: The automated AmpliSeq for Illumina NGS workflow

Table 1: Automation partners and method status

Partner	System	Single tube DNA Hotspot Panel	Single tube DNA/RNA Focus Panel	Dual tube DNA/RNA Comprehensive Panel v3	Illumina Qualified
BECKMAN COULTER	Biomek i5 Automated Workstation	✓	✓	✓	✓
eppendorf	epMotion 5075 TMX	✓	✓	✓	✓
	epMotion 5073m	✓	✓	–	–
HAMILTON	NGS STAR Liquid Handling System	✓	–	–	–
	Genomic STARlet	✓	–	–	–
PerkinElmer	Sciclone G3 Liquid Handling Workstation	✓	✓	✓	✓

Checkmark indicates the AmpliSeq for Illumina panel has been tested with the automation system. All three panels must be successfully tested to achieve "Illumina Qualified" status. Details for each automated method may be obtained from the respective vendor.

Benefits of partnership

The Illumina Automation Partnership program provides choices among the leading names in laboratory automation. Through deep collaboration, we work with our partners to develop and test Illumina Qualified methods across multiple workflows and applications. The “Illumina Qualified” designation means our analysis has shown libraries prepared with these methods perform comparably to those prepared manually. By leveraging our partnerships, customers can benefit from our library prep and sequencing knowledge and the automation expertise of our partners to ensure maximum processing efficiency and production of high-quality sequencing data (Table 1).

Scalability with automation

A key advantage of choosing an automated platform from one of our partners is the scalability of both sample number and application type. Illumina Qualified methods can run as few as eight samples at a time with the option to scale up to 96 samples in a single run (Figure 2) (Table 2). Our partners' methods also allow for parallel processing of DNA and RNA samples for supported panels (eg, AmpliSeq for Illumina Focus Panel and AmpliSeq for Illumina Comprehensive Panel v3).

Also, some researchers may start with amplicon testing for content validation then move to enrichment for longer term implementation of standardized testing. Utilizing an Illumina partner system enables additional Illumina Qualified workflows to run on an existing platform, thereby removing the requirement of additional capital expenditures to support multiple NGS workflows.

If preparing the maximum number of libraries per kit, more than one kit may be required to accommodate for higher dead volume requirements associated with automated platforms and any variation in overfill volumes by the original reagent manufacturer.

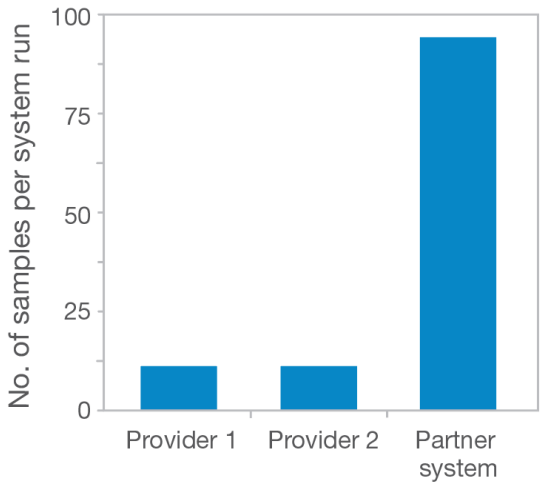

Figure 2: Sample throughput by system—Illumina automation partners provide the ability to process up to 96 samples per system run. Supported throughput represents 48 DNA and 48 RNA samples for dual DNA/RNA panels, such as AmpliSeq for Illumina Comprehensive Panel v3, or 96 DNA samples for DNA panels such as AmpliSeq for Illumina Cancer Hotspot Panel v2

Table 2: Supported sample throughputs

AmpliSeq for Illumina Cancer Hotspot Panel v2	Sample range	Sample run time[a]	Hands-on time	Manual touch points
Beckman Coulter Biomek i5 Automated Workstation	8–96	2 hr 58 min	25 min	2
Eppendorf epMotion 5075 TMX	8–96	3 hr 10 min	35 min	6
Hamilton NGS STAR Liquid Handling System	8–96	2 hr	25 min	2
Perkin Elmer Sciclone G3 Liquid Handling Workstation	8–96	2 hr 10 min	20 min	4
AmpliSeq for Illumina Focus Panel				
Beckman Coulter Biomek i5 Automated Workstation	8–96	3 hr 9 min	30 min	2
Eppendorf epMotion 5075 TMX	8–96	4 hr 10 min	40 min	8
Hamilton NGS STAR Liquid Handling System	8–96	2 hr 20 min	30 min	2
Perkin Elmer Sciclone G3 Liquid Handling Workstation	8–96	2 hr 35 min	25 min	7

a. Sample run time is for 24 samples and represents instrument processing time only and excludes cDNA synthesis, incubations, and PCR.

Simplified bioinformatics

Illumina provides various options for downstream, bioinformatic processing of NGS sample data. Users can take advantage of Local Run Manager, an on-instrument solution to create a sequencing run, monitor run status, analyze sequencing data, and view results. Various optional software modules are available to perform analysis procedures designed for different library types. Users can also access BaseSpace™ Sequence Hub, the Illumina cloud-based data storage and management tool that allows for monitoring of instrument run quality and sharing of data with collaborators on a global scale. Third-party tools developed by leading providers of informatics solutions for tertiary analysis, variant calling, and so on, are also available. Illumina is dedicated to helping customers simplify and maximize data from their NGS runs.

High-quality data

To ensure robust automated methods, three ready-to-use panels of increasing complexity/target number (HotSpot, Focus and Comprehensive v3) were tested with Coriell DNA, Horizon DNA and SeraCare samples. Final qualification of the automated method was obtained by running 48 samples with the AmpliSeq for Illumina Comprehensive Panel v3. Library QC and sequencing data were analyzed to ensure that comparable results to manual processing were obtained (Figure 3).

Additionally, automated library prep methods for the AmpliSeq for Illumina Comprehensive Panel v3 from three different partners were evaluated to recognize structural variants within RNA transcripts. Libraries prepared from the Seraseq Fusion RNA Mix v2 reference were sequenced on the MiSeq System. Results showed all three methods detected all known gene fusions within this sample (Table 3).

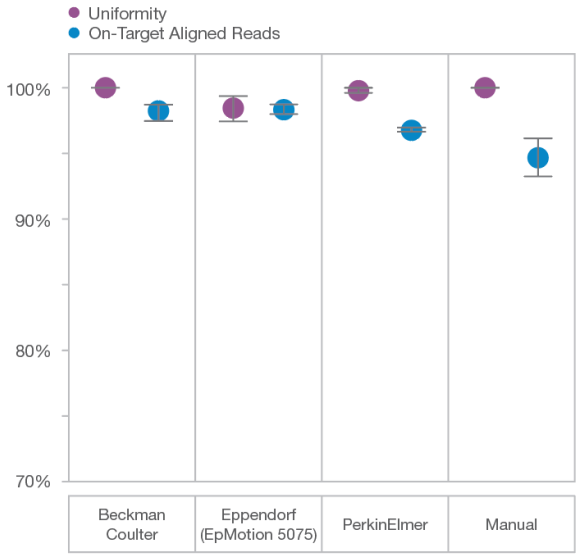

Figure 3: High coverage uniformity and on-target alignment with automated library prep—Coriell and Horizon Discovery DNA samples were used to evaluate the performance of three automation partners with the AmpliSeq for Illumina Comprehensive Panel v3. Libraries were prepared from 24 replicate samples using the indicated automation system and sequenced on the MiSeq System. Error bars indicate variability of technical replicates.

Table 3: Accurate calling of gene fusions

Fusion	Beckman Coulter	Eppendorf	Perkin Elmer
RNA Source: Seraseq Fusion RNA Mix v2			
CD74-ROS1	✓	✓	✓
EGFR-SEPT14	✓	✓	✓
EML4-ALK	✓	✓	✓
ETV6-NTRK3	✓	✓	✓
FGFR3-BAIAP2L1	✓	✓	✓
FGFR3-TACC3	✓	✓	✓
KIF5B-RET	✓	✓	✓
LMNA-NTRK1	✓	✓	✓
MET-MET	✓	✓	✓
NCOA4-RET	✓	✓	✓
PAX8-PPARG	✓	✓	✓
SLC34A2-ROS1	✓	✓	✓
SLC45A3-BRAF	✓	✓	✓
TMPRSS2-ERG	✓	✓	✓
TPM3-NTRK1	✓	✓	✓

The fusion-positive RNA sample Seraseq Fusion RNA Mix v2, was used to generate RNA libraries with the indicated automated method for the AmpliSeq for Illumina Comprehensive Panel v3 and sequenced on the MiSeq System.

Summary

Illumina has worked with its automation partners to provide scalable, automated solutions for the AmpliSeq for Illumina resequencing assays. Combining these automated solutions with the expansive portfolio of Illumina sequencing systems and cloud-based analysis tools provides a comprehensive, supported workflow for users of all experience levels. Analysis of resulting libraries from various sample types and input quantities show a robust, reproducible solution across increasing sample number and assay complexity. Illumina continues to work with its automation partners to provide a wide range of system options to meet future throughput and application requirements.

Learn more

To learn more about automation solutions, visit www.illumina.com/techniques/sequencing/ngs-library-prep/automation.html

To learn more about AmpliSeq for Illumina, visit www.illumina.com/products/by-brand/ampliseq.html

Illumina, Inc. • 1.800.809.4566 toll-free (US) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com

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