

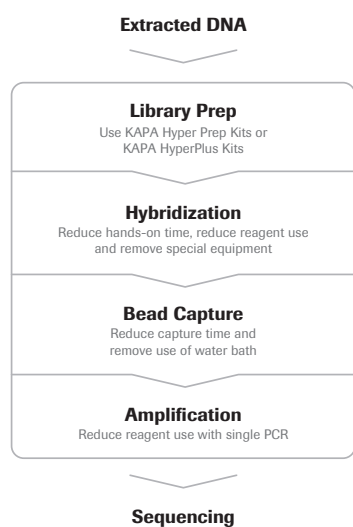
# SeqCap EZ HyperCap Workflow for Target Enrichment

## *Performance. Speed. Automatability.*

The SeqCap EZ Target Enrichment System is based on an innovative solution-based hybridization capture technology. Built upon an optimized design algorithm and manufactured with high probe density, the SeqCap EZ target enrichment products demonstrate uniform and deep coverage enabling discovery and detection of variants.

High-efficiency capture of the SeqCap EZ product\* is now supported with an additional, more efficient protocol to facilitate fast, routine, high-throughput targeted sequencing.

The SeqCap EZ HyperCap Workflow provides an integrated workflow solution that combines high-efficiency library preparation kits from Kapa with performance-optimized SeqCap capture protocol. It is an application-specific approach for efficient, streamlined and automatable targeted sequencing.



**Figure 1: Overview of SeqCap EZ HyperCap Workflow for Target Enrichment.** The SeqCap EZ HyperCap Workflow has been designed with improvements throughout the library prep and target enrichment process

The KAPA Hyper Prep and KAPA HyperPlus kits utilize novel one-tube chemistry with optimally formulated and evolved enzymes. Both kits enable higher yields of adapter-ligated library and lower amplification bias resulting in higher library diversity, lower duplication rates and more uniform coverage.

The protocol has been validated for use with both whole exome and custom SeqCap EZ Target Enrichment Systems.

### The benefits of the HyperCap protocol include:

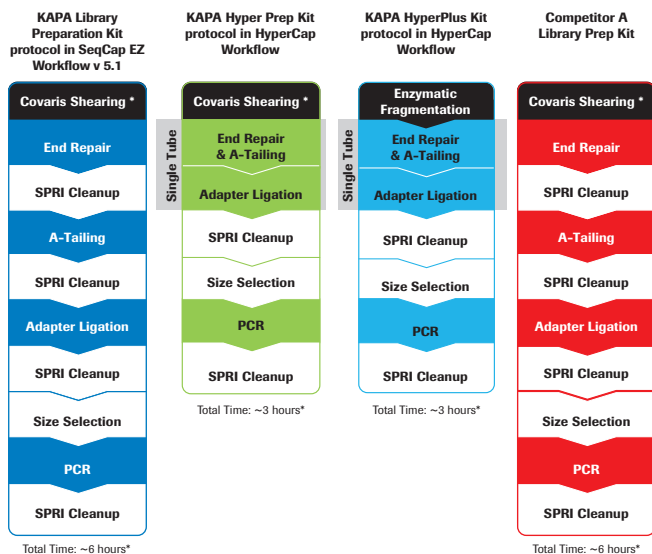
- **Speed with streamlined workflow\***
  - Greatly reduced DNA library construction time in comparison to commonly used library preparation protocols (Figure 2a)
  - Highly streamlined hybridization-based capture protocol
  - Shortened overall workflow time from DNA to sequencing in 1.5 days (Figure 2a & 2b)
- **Versatile performance for maximum flexibility**
  - Streamlined protocol demonstrating robust performance metrics for mechanical and enzymatic shearing (Figure 3, 4a, 4b and 4c)
  - Demonstrated performance across multiple sample types and design sizes\*
- **Easily automatable workflow with broad platform compatibility**
  - Reduced instrumentation requirement enabling easy automation
  - Workflow allows multiplexing, thus optimizing robot utilization

Learn more about the SeqCap EZ HyperCap User's Guide v1.0 at [sequencing.roche.com/hypercap](https://sequencing.roche.com/hypercap)

For life science research only. Not for use in diagnostic procedures.

# Speed with Streamlined Workflow

The HyperCap workflow using the KAPA Hyper Prep or KAPA HyperPlus kits allows flexibility in choosing between mechanical shearing (using Covaris) and enzymatic fragmentation as needs dictate. Overall processing time for library construction can be reduced by 50% compared to the standard KAPA Library Preparation Kit. Integration of the highly efficient, flexible single-tube KAPA Hyper Prep and KAPA HyperPlus library preparation kits, reduces library construction time to less than 3 hours (Figure 2a), making it possible to shorten the overall workflow from DNA to sequencing to 1.5 days (Figure 2b).

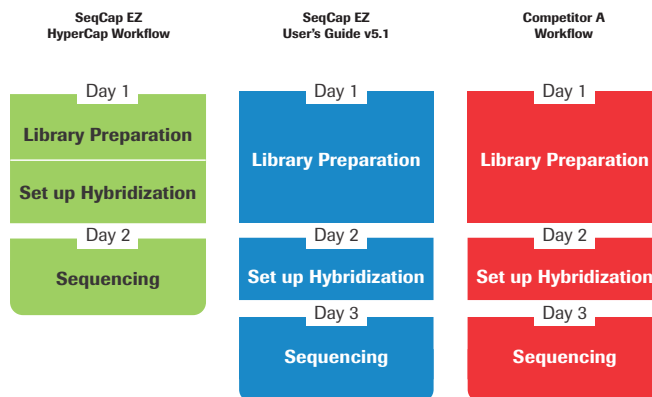


**Figure 2a: Workflow of various library preparation kits.** KAPA Hyper Prep and HyperPlus kits reduce library construction time by half.

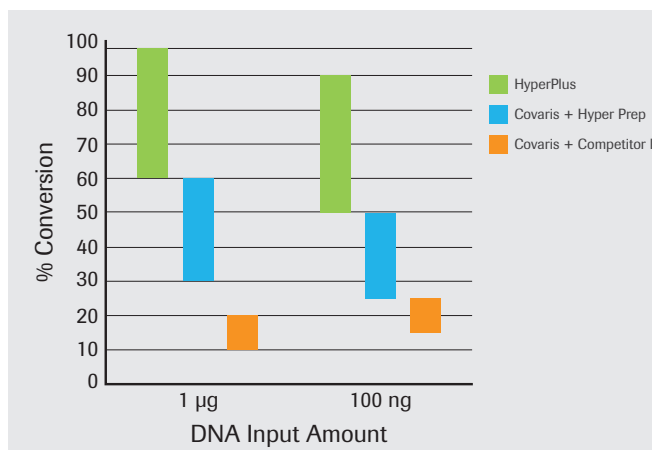
\*Covaris shearing time is dependent on instrument, make, model and batch size. Time estimate for the KAPA Library Preparation Kit and KAPA Hyper Prep Kit do not include Covaris shearing.

The capture workflow has been optimized by eliminating steps that require user intervention and/or use of specialized equipment, including speed vacuum and water bath, hence enabling easy automation. Reagent usage, incubation time and hands-on time throughout the protocol have been trimmed where appropriate with no compromise to overall performance.

The KAPA Hyper Prep and KAPA HyperPlus kits demonstrate higher conversion rates of input DNA (Figure 3) into adapter-ligated library. The resulting libraries offer greater molecular complexity translating to better enrichment metrics.



**Figure 2b: Workflow of the SeqCap EZ protocols.** By implementing the SeqCap EZ HyperCap protocol, the user can reduce the workflow time into 1.5 days - from extracted DNA to sequencing ready library.



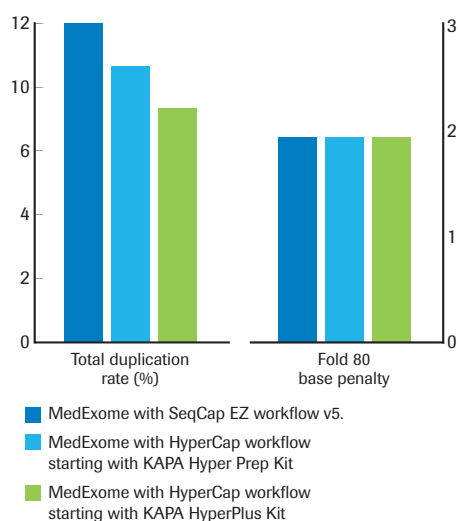
Enzymatic fragmentation with KAPA HyperPlus Kits include low-bias enzymatic fragmentation with high conversion rate (Figure 3). It allows the flexibility to further reduce workflow time with efficient and effective fragmentation for automated and high-throughput workflows.

**Figure 3: The KAPA HyperPlus Kit offers industry-leading conversion rates.** Conversion rate (% input DNA converted to sequencing-ready library) is an indication of library construction efficiency, and ultimately determines library complexity. The efficiency of ligation-based library prep decreases with input. Data represents typical results from multiple experiments.

## Versatile Performance for Maximum Flexibility

The HyperCap protocol was tested using the SeqCap EZ MedExome Kit (47 Mb) and SeqCap EZ Comprehensive Cancer Panel design (4 Mb). Analyses of performance metrics for uniformity, coverage and on-target rates were evaluated for both panels using both KAPA Hyper Prep and KAPA HyperPlus kits.

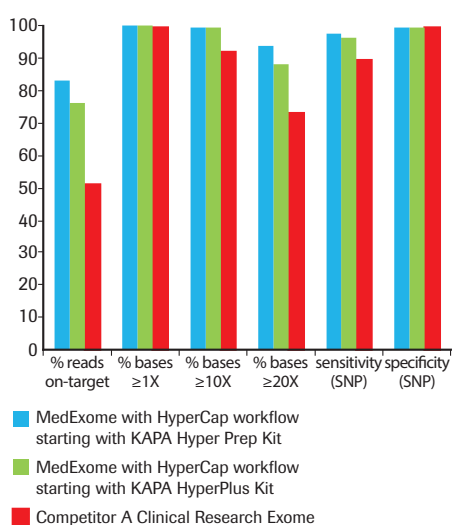
Results of testing with the SeqCap EZ MedExome and Comprehensive Cancer design showed no impact to uniformity as measured by Fold 80 Base Penalty. Fold 80 is the fold sequencing required to bring 80% of the targeted bases to the mean. High uniformity, as indicated by a low fold 80 value, decreases the cost of sequencing and increases sensitivity for variant detection, which is a key performance indicator of enrichment.



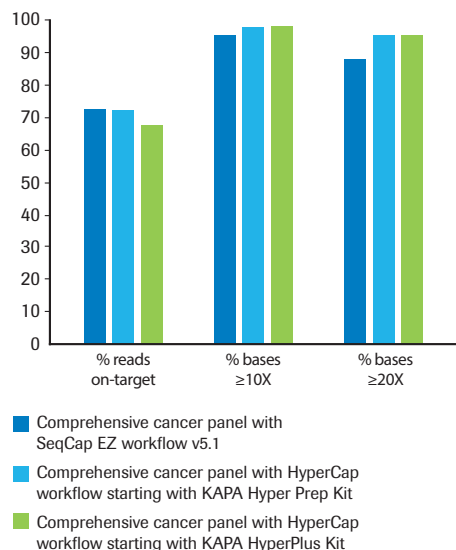
**Figure 4a: High uniformity and lower duplication rates with HyperCap Workflow.** With KAPA Hyper Prep and HyperPlus kits, the workflow provides lower duplication rates and equivalent uniformity compared to SeqCap EZ v5.1 workflow.

Both KAPA Hyper Prep and HyperPlus kits provide lower duplication rates than the standard KAPA Library Preparation Kit, indicating ability to retain molecular complexity of samples resulting in better performance metrics (Figure 4a).

KAPA Hyper Prep and KAPA HyperPlus kits showed improved coverage ( $\geq 20X$ ) over the standard KAPA Library Preparation Kit (Figure 4c). With KAPA Hyper Prep and HyperPlus kits, MedExome demonstrated same excellent performance as with SeqCap EZ v5.1 workflow, providing better data quality than competitor A's exome (Figure 4b).



**Figure 4b: HyperCap workflow leads to excellent enrichment metrics.** SeqCap EZ MedExome through the HyperCap workflow shows better coverage performance metrics than the Competitor A Clinical Research Exome.



**Figure 4c: The HyperCap workflow provides improved coverage performance over the SeqCap EZ workflow v5.1, as demonstrated by SeqCap EZ Comprehensive Cancer Panel.**

**Experimental Details for Figures 4a, 4b and 4c.** Replicate libraries were prepared from 100 ng of NA12891 Coriell DNA using the KAPA Library Prep Kit (v5.1), KAPA Hyper Prep Kit or KAPA HyperPlus Kit (HyperCap Workflow User's Guide v1.0). Six libraries were pooled for capture with SeqCap EZ MedExome or Comprehensive Cancer Panel design with overnight hybridization. Data were subsampled to 60 million reads for the MedExome kit and 4 million reads for the Comprehensive Cancer panel design, and analyzed using the standard RNG informatics pipeline.

Competitor A's Clinical Research Exome (CRE) data was generated by a 3<sup>rd</sup> party service provider using 3  $\mu$ g of Coriell NA 12891 DNA processed using XT kit following vendor recommendations. Data was subsampled to 60 million reads, and analyzed using the standard RNG informatics pipeline. Data on file with Roche.

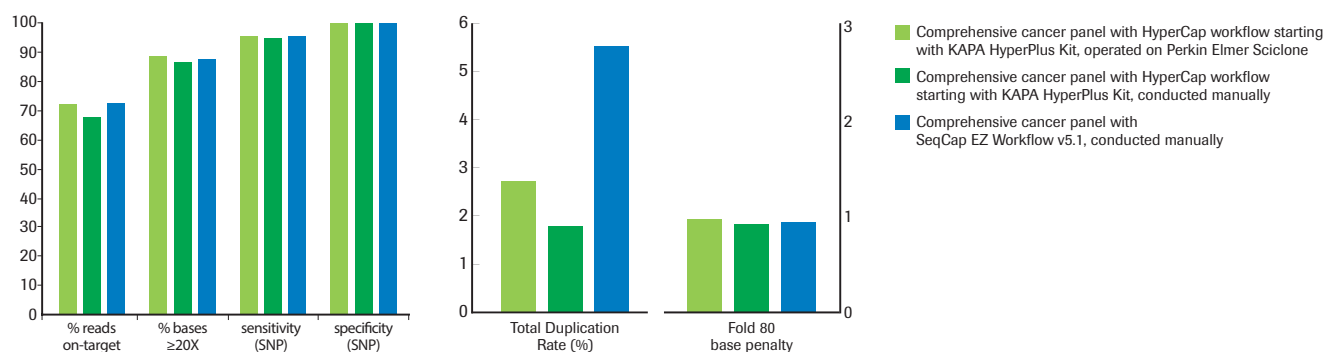
# Easily Automatable Workflow with Broad Platform Compatibility

The SeqCap EZ HyperCap Workflow offers a streamlined, automation-friendly capture protocol that minimizes user intervention and eliminates the use of specialized, off-deck equipment such as speed vac, water bath, centrifuge and vortex mixer, saving hands-on time. The protocol offers automation capability across multiple platforms.

Coriell samples were enriched using the SeqCap EZ Comprehensive Cancer Panel design on Perkin Elmer Sciclone NGSx platform following SeqCap EZ HyperCap Workflow v1.0 (with

minor process deviations). Results from the automated HyperCap workflow were concordant with manual methods (Figure 5).

The SeqCap EZ HyperCap Workflow integrates KAPA Hyper Prep and HyperPlus kits with further optimized SeqCap EZ target enrichment protocol, enabling a powerful solution that can be tailored to different applications, sample types, and operational objectives. This workflow reduces hands-on and overall time, user intervention, and cost, all while maintaining excellent sequence data quality.



**Figures 5: The automated HyperCap workflow showed concordant performance as the manual method.** Six replicate libraries were prepared from 100 ng of DNA using the KAPA HyperPlus Kit. Minor process deviations were made to the HyperCap workflow to optimize performance on the automated platform. Results were then compared to replicate libraries of NA12891 processed through the manual HyperCap workflow and standard SeqCap EZ SR User's Guide v5.1. Sequencing data were subsampled to 4 million reads, and analyzed using the standard RNG informatics pipeline.

SeqCap EZ Exome Products	Kits and Reagents** continued...
SeqCap EZ MedExome Target Enrichment Kit	SeqCap Hybridization and Wash Kit
SeqCap EZ MedExome Plus Target Enrichment Kit	SeqCap EZ Reagent Kit v2
SeqCap EZ Exome v3 Target Enrichment Kit	SeqCap EZ Reagent Kit v2 Plus
SeqCap EZ Exome v3 +UTR Target Enrichment Kit	
SeqCap EZ Exome Plus	
Kits and Reagents**	SeqCap EZ Custom Products
KAPA Hyper Prep Kit**	SeqCap EZ Choice & EZ Choice XL
KAPA HyperPlus Kit**	<b>SeqCap EZ Choice Library</b> (Target 100 Kb - 7 Mb human (HG18 or HG19) genomic regions)
SeqCap Adapter Kit A	Comprehensive Pan-Cancer Panel Design
SeqCap Adapter Kit B	Neurology Gene Panel Design
SeqCap Pure Capture Bead Kit	50 MB UTR Design
SeqCap HE-Oligo Kit A	Major Histocompatibility Complex (MHC) Design
SeqCap HE-Oligo Kit B	SeqCap EZ Choice XL Library (Target 7 Mb - 50 Mb human (HG18 or HG19) genomic regions)
SeqCap EZ Accessory Kit v2	<b>SeqCap EZ Developer Library</b> (Target up to 200 Mb of genomic regions, contiguous or noncontiguous, for any sequenced genome)
	Mitochondrial Genome Design



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\*Clark M, et al. Nat. Biotech. 2011; Asan XY, et al. Genome Biol. 2011 Sep 28;12(9):R95.

\*Data on file

\*\*Complete list of reagents can be found on [sequencing.roche.com](http://sequencing.roche.com)

\*\*Availability of KAPA Hyper Prep and HyperPlus kits vary by region and country. Please check with your Roche representative

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