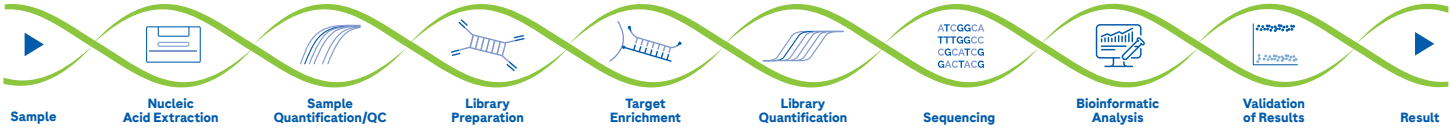
Targeted DNA Sequencing

Seq success with every sample



Targeted DNA Sequencing

Next-generation sequencing (NGS) and bioinformatic analysis

Every sample is precious

Targeted DNA sequencing enables researchers to dedicate their sequencing budget to selected genomic regions—such as specific genes, regulatory regions, exons, or intergenic regions—thus minimizing wasted sequencing reads.

Hybridization-based target enrichment for next-generation sequencing (NGS), also known as capture-based target enrichment, can be used to identify single nucleotide polymorphisms (SNPs), insertions/deletions (indels), copy number variation (CNV), and structural variants. Effective enrichment of desired regions is enabled by the use of carefully designed probes that are complementary to the target regions.

Primer Extension Target Enrichment (PETE) is a novel capture technology designed to combine both hybrid capture and primer extension reactions to capture and release targeted library molecules for sequencing. This workflow preserves the performance of conventional hybridization while enabling a more efficient workflow. PETE is designed and optimized for small design sizes and validated to detect all major somatic variants in cfDNA, FFPE, and RNA samples– including single nucleotide variants (SNVs), short indels, CNVs, microsatellite instability (MSI), and fusion transcripts (novel and known) and is especially suited for small-panel oncology research applications.

- **Leverage more than a decade of design experience** to create your custom probes pools, using the easy-to-use HyperDesign online tool
- **Enrich regions of interest with pre-designed or custom probe panels**, for either hybridization-based or PETE-based panels
- **Streamline target enrichment** using the updated KAPA HyperCap Workflow, driven by KAPA HyperPrep Kit (mechanical fragmentation) or KAPA HyperPlus Kit (enzymatic fragmentation) for library preparation
- **Receive industry leading support** by using the our complete sample prep workflow for NGS, including automated DNA extraction, library preparation for sequencing on Illumina platforms, and accurate qPCR-based QC of input DNA and final sequencing libraries

Scan to learn more

Nucleic Acid Extraction

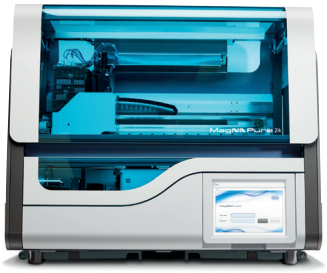
MagNA Pure 24 and MagNA Pure 96 Systems

High-quality starting material increases sequencing success. High-molecular weight input DNA is essential for the creation of libraries with the 350 – 650 bp inserts required for sequencing whole human genomes on Illumina® sequencers.

Obtain high-quality, high-molecular-weight DNA with the **MagNA Pure 24** and **MagNA Pure 96 Systems**. These fully automated nucleic acid extraction instruments provide walkaway automation, require less user intervention, and minimize variability between extractions.

- Reliable DNA extraction from as little as 200 µL whole blood
- Scalable extraction for low, mid, or high throughput
- cfDNA protocols compatible with next-generation sequencing (NGS) workflows

MagNA Pure 24 System



MagNA Pure 96 System

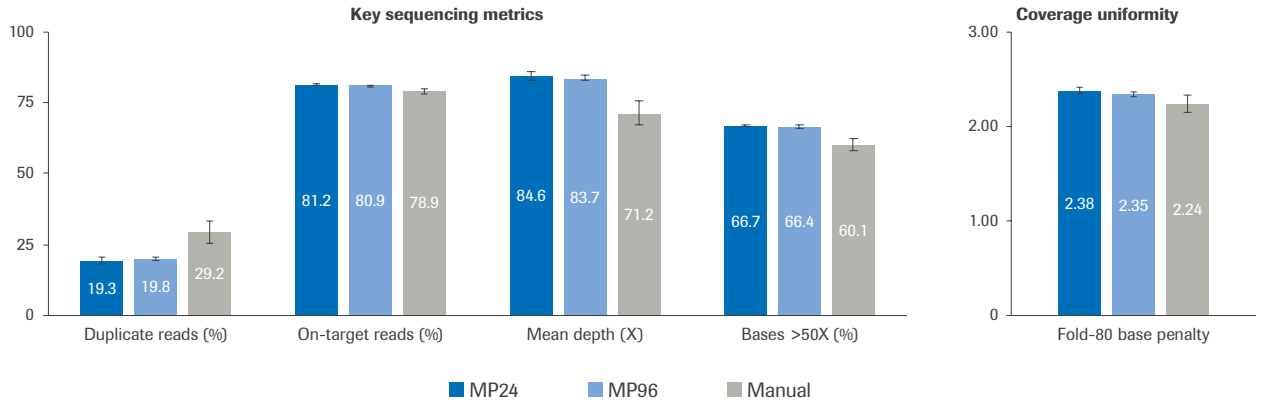


Figure 1. cfDNA extracted from MagNA Pure 24 and MagNA Pure 96 Systems yields superior sequencing coverage and fewer duplicate reads compared to manual extraction. Target-enriched libraries were prepared from cfDNA isolated from 4 mL of plasma, using either the MagNA Pure 24 cf ds 4000 hp protocol, the MagNA Pure 96 cfdna ds 4000 protocol, or a manual protocol. The total yield of cfDNA for each sample was used as input into the HyperCap 2.0 workflow, using the KAPA HyperPrep Kit and the SeqCap EZ Human Oncology Panel (2.75 Mb). Prior to target capture, libraries were multiplexed; each capture contained samples obtained from each of three extraction workflows. Sequencing was performed on the NextSeq 500 (2 x 75 bp). Raw reads were randomly downsampled to 6M prior to analysis. Each bar represents the mean of 5 replicate extractions; error bars indicate the standard deviation.

Table 1. Overview of MagNA Pure System protocols optimized for double-stranded DNA.

Platform	Sample input	Nucleic acids output	Protocol
MagNA Pure 24	blood	genomic DNA	hgDNA ds 200
	plasma	cell-free DNA	cfNA ds 2000
	plasma	cell-free DNA	cfNA ds 4000 hp
MagNA Pure 96	blood	genomic DNA	DNA Blood SV
	plasma	cell-free DNA	cfNA ds 2000
	plasma	cell-free DNA	cfNA ds 4000

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DNA Library Preparation

KAPA DNA Library Preparation Kits

KAPA DNA library prep kits deliver high performance across a diverse array of experimental conditions and sample inputs. Workflows are made simple with sample-conserving, automatable protocols that free up valuable hands-on time while delivering reproducible, high-quality results. Choose from PCR-free or with-PCR formats (including our low-bias, high-fidelity KAPA HiFi DNA Polymerase) for mechanical or enzymatic fragmentation methods.

The new KAPA EvoPrep and KAPA EvoPlus V2 Kits enable you to:

- Achieve higher yields and library conversion efficiency across a range of sample types and DNA inputs (as low as 100 pg);
- Increase efficiency and convenience with automation-friendly ReadyMix reagents and KAPA T4 Ligase, in plated or tube format;
- Rely on consistent performance of kits manufactured to meet the highest quality standards;
- Preserve library diversity with minimal bias for a seamless transition into downstream applications.

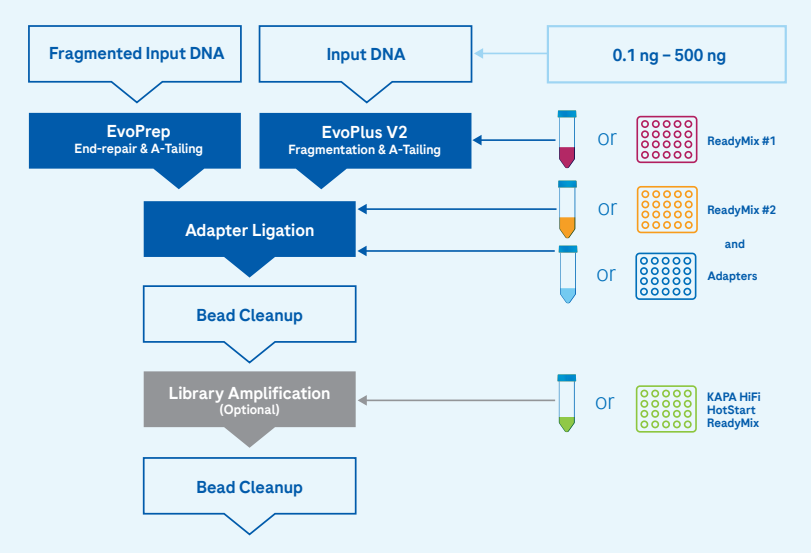

Figure 1. Overview of the newest KAPA DNA library preparation kits, KAPA EvoPrep Kit and KAPA EvoPlus V2 Kit. (KAPA HyperPure beads, KAPA UDI Adapter Kits, and KAPA Library Amplification Primer Mix (10X) or KAPA HyperPlex Adapters sold separately).

Table 2. Comparison of KAPA library preparation kits for DNA.

	Simplify high-performance library prep from mechanically fragmented DNA starting with low inputs. **KAPA EvoPrep Kits**	**Convert** more unique fragments from mechanically fragmented inputs, including low-quality DNA & cfDNA. **KAPA HyperPrep Kits**	**Reduce** workflow complexity with inhibitor-tolerant formulas, leading to more consistent results. **KAPA EvoPlus V2 Kits**	**Save time** with a single-tube workflow, for high yields from low-quality inputs such as FFPE. **KAPA HyperPlus Kits**
Fragmentation method	Mechanical	Mechanical	Enzymatic	Enzymatic
Hands-on time	Better	Good	Best	Better
Inhibitor tolerance	Broad tolerance, including EDTA	Robust	Broad tolerance, including EDTA	Low; EDTA-sensitive
Sample input range	0.1 ng – 500 ng	1 ng – 1000 ng	0.1 ng – 500 ng	1 ng – 1000 ng
PCR and PCR-free formats	24, 96, 384 rxn tubes & 96 rxn plates	8, 24, 96 rxn tubes	24, 96, 384 rxn tubes & 96 rxn plates	8, 24, 96 rxn tubes

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NGS Sample Prep Automation

The AVENIO Edge Liquid Handling System for end-to-end library prep and target enrichment

Automate your low- to medium-throughput NGS library preps with as little as 20 minutes of hands-on time and no prior NGS or automation experience.

Freedom to walk away with confidence and trust in the results

AVENIO Edge Liquid Handling System is Roche's fully automated solution for NGS library preparation, including target enrichment and library normalization and pooling. It is designed to greatly reduce the complexity of automation for users at any level, and provide a true walk-away experience.

As little as 20 minutes of set up time for each run

On-deck thermocycling and quantification module

Cartridge-based and ready-to-use reagents

Intuitive software and a built-in controller PC

Remote access connectivity to enable real-time troubleshooting

Glove-compatible touchscreen

Easily run your whole exome or smaller cancer research panels on the **AVENIO Edge Liquid Handling System** with as little as 20 min hands-on time for end-to-end library prep and target enrichment.

Examples:

- KAPA HyperExome V2 Probes
- KAPA HyperCap Non-Hodgkin Lymphoma (NHL) Panel
- KAPA HyperCap Oncology Panel

For more information about the AVENIO Edge Liquid Handling System, contact your Roche sales representative or visit: **sequencing.roche.com/AVENIOEdge**

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Automated KAPA NGS Library Prep Workflows

On non-Roche liquid handlers

Roche's NGS Automation Support Team, in collaboration with non-Roche liquid handling vendors, creates menus of automated methods for KAPA library prep reagents.

Let's talk about the next steps to get you up and running with automating your NGS library prep protocols.

Scan the QR code below or fill out the Contact Us form at **go.roche.com/AutomationSupport**.

Table 3. Automated NGS library preparation workflows supported by Roche.

Vendor and Platform		DNA Library Preparation				RNA Library Preparation					Quant and QC	Target Enrichment	Oncology
Vendor	Platform	KAPA HyperPrep	KAPA HyperPlus	KAPA EvoPlus		KAPA Stranded RNA-seq	KAPA Total RNA HyperPrep	KAPA mRNA HyperPrep	KAPA RNA HyperPrep RiboErase +Globin	KAPA Shortened RNA HyperPrep RiboErase +Globin	KAPA Library Quant	KAPA HyperCap v3	AVENIO ctDNA
				Tubes	Plates								
Agilent	Bravo NGS	✓	✓			✓					✓		
	Bravo NGS Workstation	✓	✓			✓	✓	✓	✓	✓	✓	✓	
Beckman Coulter Life Sciences	Biomek FX^P Hybrid Workstation	✓	✓			✓	✓	✓	✓		✓	✓	✓
	Biomek i7 Hybrid Workstation	✓	✓	✓	✓		D	✓	✓	✓	✓	✓	
	Biomek i5 MC Workstation	✓	✓		✓								
	Biomek i5 Span-8 Workstation	✓	✓										
Eppendorf	epMotion® 5075t	✓	✓			✓	✓	✓	✓		✓	D	
Hamilton	NGS STAR (8-channel)	✓	✓					✓	✓		✓	✓	
	NGS STAR MOA	✓	✓	D									
	NGS STAR V				✓								
Revvity	Sciclone G3 NGSx Workstation	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓
	Sciclone G3 NGSx IQ Workstation	✓	✓									✓	
	Zephyr G3 NGS Workstation	✓	✓			✓	✓	✓	✓		✓		
SPT Labtech	firefly®				✓						✓		
Tecan	DreamPrep® NGS	✓	✓										
	Freedom EVO® NGS Platform	✓	✓				✓	✓	✓				

(✓) = Complete and in distribution (Roche) (D) = In development (Roche) ✓ = Complete and in distribution (Vendor) **D** = In development (Vendor)

Agilent BECKMAN COULTER Life Sciences eppendorf HAMILTON revvity sptlabtech TECAN.

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Target Enrichment

KAPA HyperCap Probes for Hybridization-based Target Enrichment

Better by Design

Combining nearly two decades of probe-design experience with an improved manufacturing process, **KAPA HyperCap Probes** offer fully customizable or pre-designed target enrichment panels for hybridization-based capture in NGS workflows. KAPA HyperCap Probes are manufactured using KAPA HiFi DNA Polymerase and are validated by NGS, resulting in high-quality, expertly designed probes to assist with your most challenging workflows.

Ready-to-ship, **pre-designed KAPA HyperCap Fixed Panels** enable faster access to relevant content, and include KAPA HyperExome V2, KAPA HyperCap Oncology Panel, and KAPA HyperCap Heredity Panel. Additional designs are available from our **KAPA HyperCap Design Share Panel collection**—developed in collaboration with leading researchers around the world—and include panels for hereditary conditions, oncology, and metabolic disease research.

Custom target enrichment panels are easily designed using the **HyperDesign** online software tool (see below). HyperDesign can be used to create either human designs (KAPA HyperChoice Probes) or non-human designs (KAPA HyperExplore Probes).

Combine KAPA HyperCap Probes with KAPA Library Preparation Kits to:

- Reduce sequencing costs and save time with superior capture uniformity.
- Reliably enrich challenging, previously inaccessible genomic regions.
- Streamline your target enrichment workflows, taking advantage of already-developed automated methods on a variety of liquid handlers, including the AVENIO Edge Liquid Handling System by Roche.

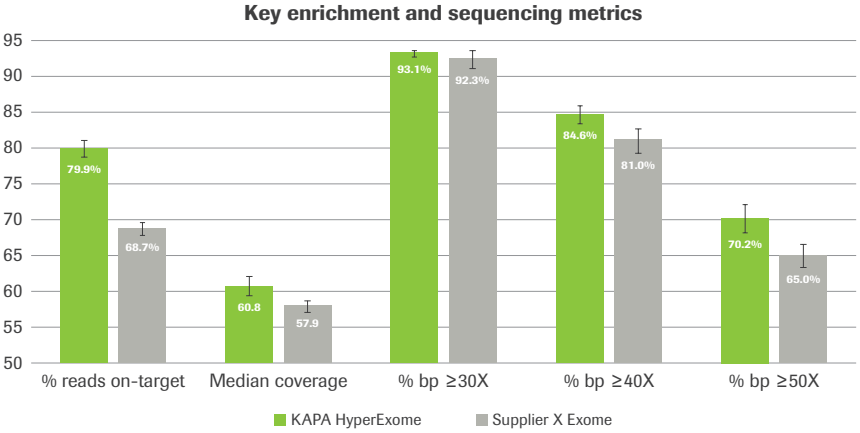

Figure 2. KAPA HyperExome yields greater % reads-on-target, deeper median coverage, and broader target coverage compared to the Supplier X exome. DNA from 16 cell lines was processed in triplicate (48 libraries per workflow); input DNA was enzymatically sheared; samples were pre-capture multiplexed in sets of 8 and hybridized for 16 hours; final post-capture libraries were amplified with 8 PCR cycles; and libraries were sequenced (2 x 100 bp) on an Illumina® NovaSeq™ sequencer. For analysis, sequencing data was subsampled proportionally to exome panel size to achieve the same targeted average depth of coverage.

Reliably enrich challenging, previously inaccessible genomic regions

The user-friendly, online HyperDesign tool builds on two decades of in silico design experience to select probe panels that achieve deeper, more uniform downstream sequencing coverage with fewer sequencing reads—even across difficult-to-capture regions.

HyperDesign

Design your new probe panel in 4 easy steps:

1. Visit **www.HyperDesign.com** and select your organism of interest.
2. Add your targets by uploading gene names, bed files, or genomic coordinates—or choose from a broad list of commonly used gene identifiers.
3. Fine-tune your inputs, review your targets, and confirm your results.
4. Submit your design for probe selection.

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Target Enrichment

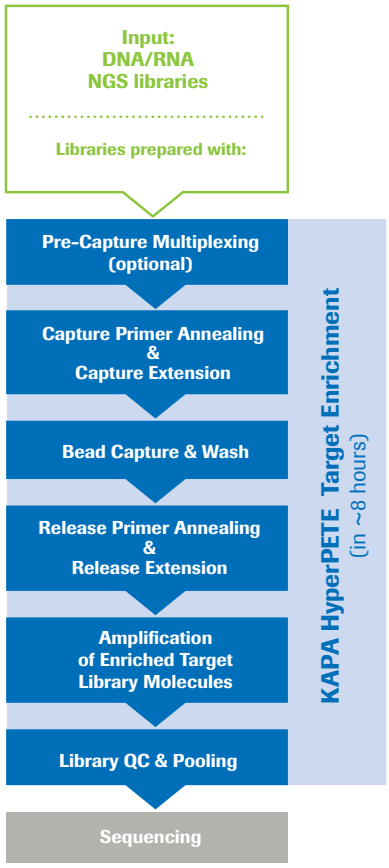
KAPA HyperPETE Primers for Primer-Extension Target Enrichment

Combine the performance of hybrid-capture target enrichment with the speed and simplicity of amplicon workflows.

KAPA HyperPETE is a novel hybrid-capture technology designed to employ primer extension reactions to specifically capture and release target library molecules for sequencing. It is designed and optimized to detect all major somatic variant types, including SNVs, short indels, CNVs, MSI, and fusion transcripts (known and novel). KAPA HyperPETE is compatible with a wide variety of sample types, including challenging samples—such as cfDNA and FFPET-derived DNA and RNA.

The KAPA HyperPETE Portfolio includes readily available fixed-design panels for hereditary oncology, oncology hotspots, lung cancer fusion variants, and pan-cancer variants (with an MSI module). In addition, custom panels can be designed using **HyperDesign**, our easy-to-use online design tool.

- Save valuable time with an efficient, single-day (~8 hours), automation-friendly workflow.
- Achieve superior performance and coverage uniformity.
- Uncover critical genomic information from a wide variety of sample types, including FFPET and cfDNA.
- Take hours off of total workflow time compared to typical hybridization capture, with time requirements similar to amplicon and anchored multiplex methods **(Figure 3)**.
- Enrich for long contiguous regions, using fewer tubes per sample compared to amplicon workflows.



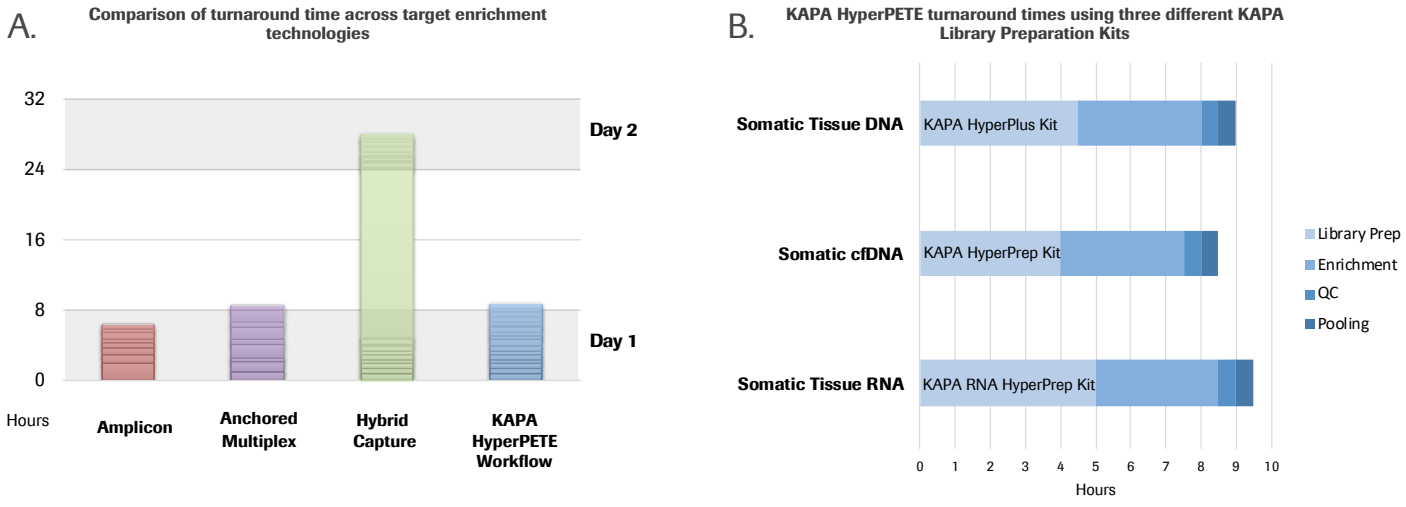


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Figure 3. The turnaround time (TAT) for KAPA HyperPETE target enrichment is similar to the TAT for amplicon-based workflows. (A) While most hybridization-based workflows take two days to complete, KAPA HyperPETE workflows can be completed in one day. **(B)** Differences in the TAT for various applications of KAPA HyperPETE are dependent on the library preparation kit used, as each kit requires slightly different completion times. However, once the libraries are created, the enrichment workflow is the same across applications.

Target Enrichment

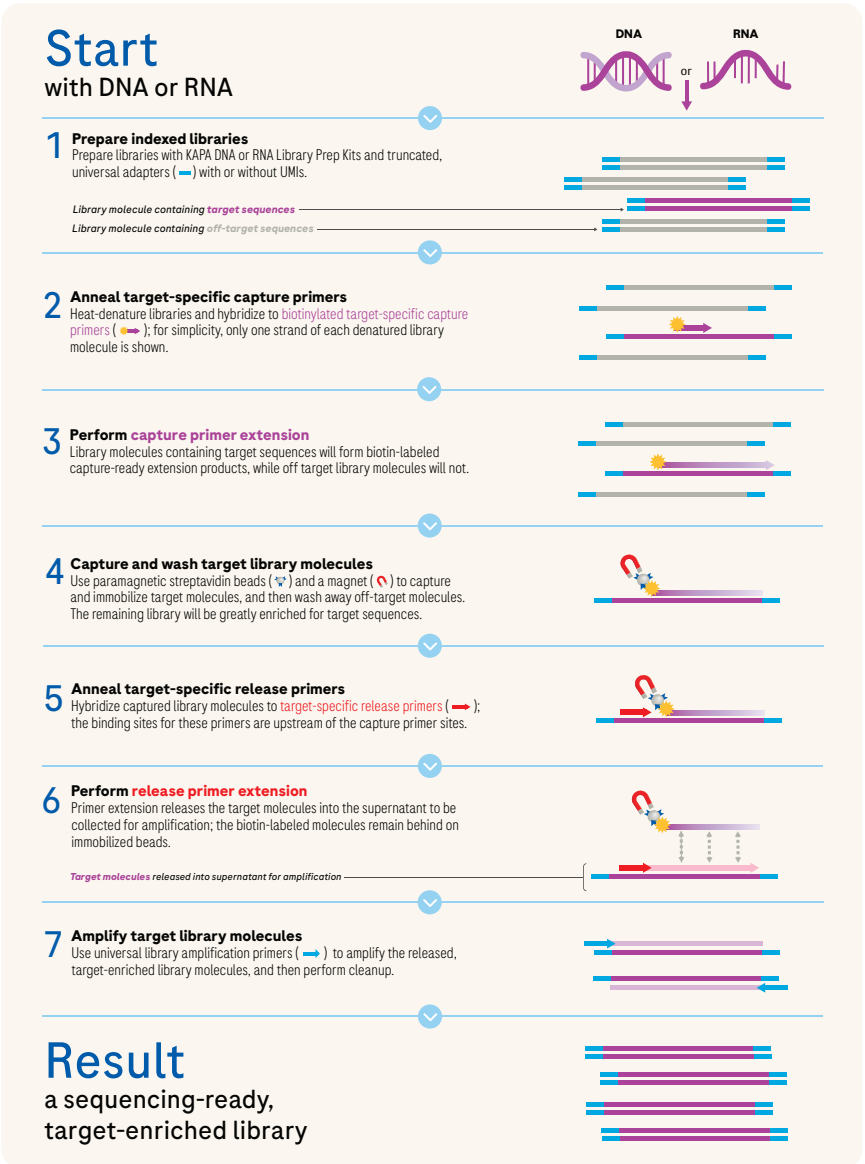
What is Primer Extension Target Enrichment (PETE)?

KAPA HyperPETE is a novel NGS hybridization capture technology designed to employ primer extension reactions to specifically capture and release target library molecules for sequencing.

What's different about PETE?

Other target enrichment technologies offer either uniform, high-quality data (via probe hybridization) or fast, simple workflows (via amplicon-based enrichment). PETE brings together the benefits of both workflows—combining speed and simplicity with deep, uniform, high-quality coverage.

Here's how KAPA HyperPETE works...



Perform fewer manual steps

Save valuable time

Achieve superior performance

The **single-day KAPA HyperPETE workflow** can detect all major somatic variant types—including SNVs, short indels, CNVs, MSI, and fusion transcripts—from a wide variety of sample types, including degraded DNA and RNA. To learn more about PETE technology and Roche's KAPA HyperPETE portfolio, visit **go.roche.com/KAPAHyperPETE**.

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Target Enrichment

From Design to Capture

Design custom NGS target enrichment panels with ease

Generating custom probe pools for targeted enrichment of next-generation sequencing libraries can be a daunting endeavor. Many traditional design tools are clunky, rely on poorly optimized design algorithms, or lack the support of a live person with years of design experience—leaving you wondering if you have the best design to capture your specific regions of interest.

Roche's online design tool, **HyperDesign**, is here to address those concerns.

HyperDesign is a user-friendly online probe design tool that takes advantage of Roche's extensive experience with *in silico* probe design. HyperDesign's proprietary design algorithm has been optimized to achieve deeper and more uniform downstream sequencing coverage with fewer sequencing reads—even for difficult-to-capture regions.

And remember—at any point in the design process, you can reach out to our **Expert Designers** for advice.

Start your new custom design

Visit **www.HyperDesign.com**, log in to your homepage, click on "new design," and ***follow these 4 simple steps...***

1. **Select your organism** of interest and name your design
2. **Add your targets** by uploading gene names, bed files, or genomic coordinates; or choose from a broad list of commonly used gene identifiers
3. **Fine-tune your inputs,** review your targets, and confirm your results
4. **Submit** your design for selection

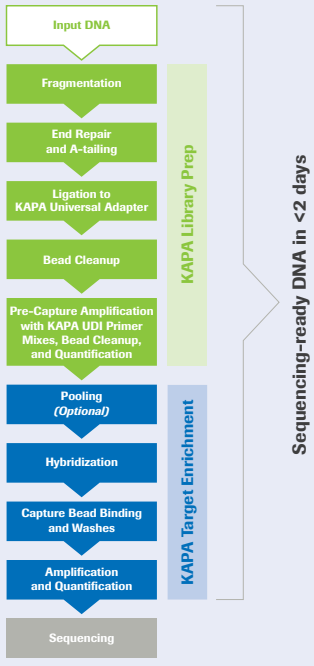
...then let our advanced algorithm do the work. Once probe selection is complete, you'll be able review the coverage results across your target regions.

Workflow focus:

KAPA HyperCap Workflow featuring KAPA HyperCap Target Enrichment probes

KAPA HyperCap Workflow delivers complex libraries by combining the high conversion rate of KAPA HyperPrep or KAPA HyperPlus Kits with KAPA Target Enrichment, creating a streamlined, single-vendor-supported workflow.

- **Achieve greater success with low-input and poor-quality samples** with KAPA HyperPrep and KAPA HyperPlus Library Preparation Kits
- **Multiplex up to 16 samples in the same capture**, and potentially post-capture multiplex more samples in the same sequencing lane, with KAPA Unique Dual-Indexed Adapters (UDI) Primer Mixes,1-384
- **Reduce workflow complexity and hands-on time** with KAPA Universal Enhancing Oligos, eliminating the need for adapter-matched blocking oligos
- **Automate the entire KAPA HyperCap Workflow v3 without the need for a SpeedVac™**—now with all hybridization and bead wash steps at 55°C



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Library Quantification

KAPA Library Quantification Kits and Roche LightCycler® Systems

Sequencing capacity is maximized when sequencing-competent molecules are accurately measured with qPCR, enabling libraries to be pooled at the desired ratios.

Clustering can be optimized by quantification of library pools, further improving sequencing results.

Roche LightCycler® 96 Instrument, LightCycler® 480 System, and LightCycler® PRO System ensure reproducible, reliable, accurate data.

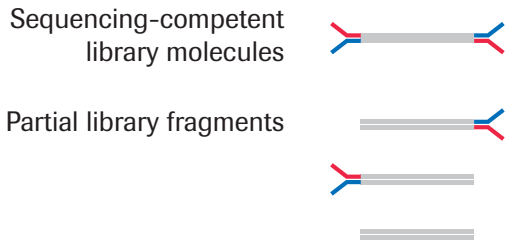
- Scalable instrument options
- Dependable temperature accuracy and homogeneity
- Ideal for use with KAPA Library Quantification Kits

KAPA Library Quantification Kits, which are referenced in thousands of scientific publications, contain all reagents needed for qPCR-based quantification of NGS libraries for Illumina® sequencing.

- Accurately quantify sequencing-competent libraries **(Figure 4)**.
- Pool libraries with better accuracy for more balanced multiplexing.
- Automate KAPA Library Quantification Kits for increased throughput.

A.

PCR-free workflow



B.

Workflow with amplification

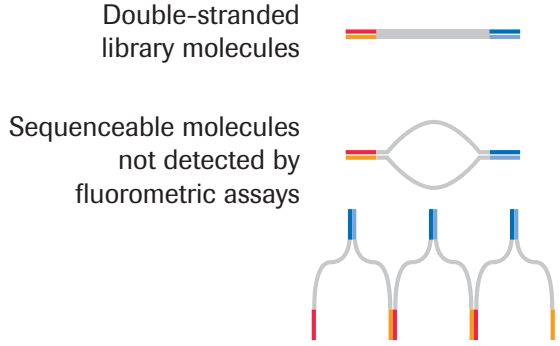


Figure 4. Library quantification via qPCR-based methods, such as the KAPA Library Quantification Kit, enables accurate sample pooling and optimal clustering.

(A) Libraries prepared with PCR-free workflows can contain partial library fragments that are not sequenceable. qPCR-based library quantification methods detect only the sequencing-competent molecules. In contrast, other assays detect fragments that are not sequenceable, leading to *underclustering* on the sequencing flow cell.

(B) Libraries prepared using methods with PCR amplification can include sequencing-competent single-stranded configurations. qPCR-based library quantification data counts these molecules. In contrast, other methods do not detect these molecules, leading to *overclustering* on the sequencing flow cell.

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For more information, please visit: **go.roche.com/target-enrichment**

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