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

DNA Extraction

MagNA Pure 24 and MagNA Pure 96 Systems

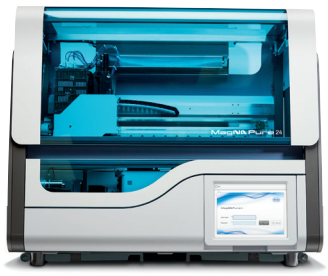
Why do extraction methods matter?

High-quality starting material leads to 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® HiSeq® and NovaSeq™ instruments.

Obtain high-quality, high-molecular-weight DNA for direct use in sequencing 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 levels
- Optimized protocols for NGS workflows with blood, plasma, or FFPET samples
- Superior sequencing coverage and fewer duplicate reads compared to manual extraction (**Figure 1**)

MagNA Pure 24 System



MagNA Pure 96 System

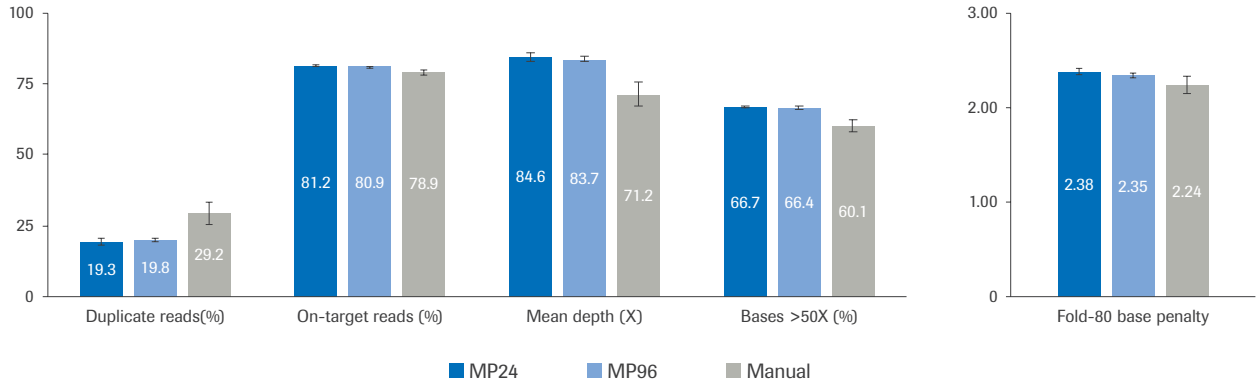


Figure 1. cfDNA extracted from MagNA Pure 24 and MagNA Pure 96 yields superior sequencing coverage and fewer duplicate reads compared to manual extraction. Target-enriched libraries were prepared from cfDNA isolated from 4 mL of plasma using either the MagNA Pure 24 cf ds 4000 hp protocol, the MagNA 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 NGS-compatible MagNA Pure System workflows

Platform	Sample input	Nucleic acids output	Protocol
MagNA Pure 24	blood	genomic DNA	hgDNA ds 200
	plasma	cell-free DNA	cfNA ds 4000 hp
	FFPET	FFPET DNA	DNA FFPET 1000
MagNA Pure 96	blood	genomic DNA	DNA Blood ds SV

DNA Library Preparation

Streamlined options for DNA library preparation

Two automation-friendly options for library preparation

Choose from mechanical or enzymatic methods for fragmenting input DNA. The choice of fragmentation method offers additional flexibility to meet the needs of each experiment (**Figure 2**).

Generate libraries from a wide variety of DNA input types. KAPA HyperPrep and KAPA HyperPlus Kits produce high-quality libraries from diverse inputs, including challenging samples such as FFPE DNA and samples with GC- or AT-rich content.

Ensure high library complexity with efficient library conversion. The robust chemistries of KAPA HyperPrep and KAPA HyperPlus Kits lead to greater conversion of input DNA into adapter-ligated molecules, improving target coverage following enrichment and reducing duplicate reads (**Figure 3**).

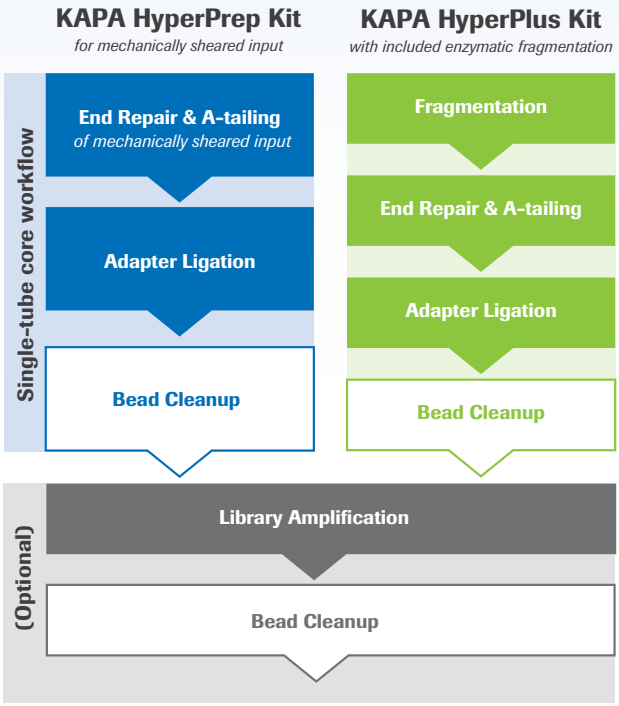


Figure 2. Summary of KAPA HyperPrep and KAPA HyperPlus Kit workflows. Both KAPA HyperPrep and KAPA HyperPlus Library Preparation Kits offer fast, streamlined workflows that are easily completed in under 3 hours. In target enrichment workflows, such as KAPA HyperCap Workflow, a double-sided size selection step using KAPA HyperPure Beads may be included after the post-ligation cleanup step.

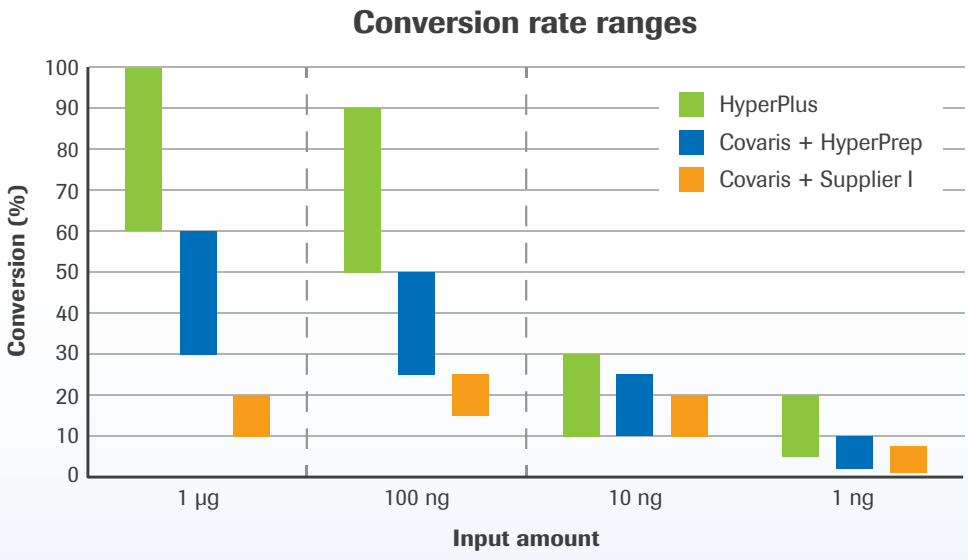


Figure 3. The KAPA HyperPlus and HyperPrep Kits demonstrate superior conversion of input DNA into adapter-ligated, sequenceable molecules. Conversion rates are highest for the KAPA HyperPlus Kit (with enzymatic fragmentation) for both high- and low-input applications. KAPA HyperPrep, which uses Covaris-sheared DNA as input, also outperforms the Supplier I kit.

NGS Sample Prep Automation Solutions

Maximize the potential of your lab with a trusted NGS partner

Automation of NGS sample prep can help your lab generate more reproducible results, increase operational efficiency, and unlock more hands-off time and resources for collaborations and innovation.

Freedom to walk away with confidence and trust in the results

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

Only 20 minutes of setup time required for each sample run

On-deck thermocycling and quantification module

Ready-to-load and barcoded KAPA reagents

Intuitive software and a built-in controller PC

Glove-compatible touchscreen

Remote access connectivity to enable real-time troubleshooting

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

AVENIO Edge System is a Class 1 IVD instrument and is intended for downstream diagnostic applications.
AVENIO Edge reagents and workflows are for Research Use Only.
They are not intended for diagnostic applications.

Automated KAPA NGS Workflows

On non-Roche platforms

Automation Support Team

Our dedicated NGS Automation Support Team works with customers to create standard KAPA protocols through collaborative partnerships with major automation vendors.

Modular designs
for workflow flexibility, enabling the inclusion of safe stopping points

User-friendly interfaces
with variable workflow options for a better user experience

Easy-to-understand variables
ideal for automation users of all experience levels

Complimentary service and support
for Roche-distributed methods

Table 2. Automated NGS library preparation workflows supported by Roche

Vendor and Platform		Library Preparation and QC								Target Enrichment	Oncology
Vendor	Platform	KAPA HyperPrep	KAPA HyperPlus	KAPA Stranded RNA-seq	KAPA Total RNA HyperPrep	KAPA mRNA HyperPrep	KAPA RNA HyperPrep RiboErase + Globin	KAPA Library Quant	KAPA hgDNA QQC	KAPA HyperCap Workflow	AVENIO ctDNA
Perkin Elmer	*Sciclone G3 NGSx*	✔	✔	✔	✔	✔	✔	✔	✔	✔	✔
	Sciclone G3 NGSx iQ	✔	✔							✔	
	JANUS G3 NGS Express	✔	✔	✔			✔	✔	✔		
	Zephyr G3 NGS	✔	✔	✔	✔	✔	✔	✔	✔		
Beckman Coulter Life Sciences	*Biomek FX^P Hybrid*	✔	✔	✔	✔	✔	✔	✔			✔
	Biomek i7 Hybrid	✔	✔		D	✔	✔	✔		D	D
	Biomek i5 MC96	✔	✔								
Hamilton	*NGS STAR (Span-8)*	✔	✔			✔	✔	✔		✔	
	Microlab VANTAGE					✔					
Tecan	*Freedom EVO NGS*	✔	✔		✔	✔	✔				
Agilent	*Bravo NGS Workstation (Option A)*	✔	✔	✔				✔			
	Bravo NGS Workstation (Option B)	✔	✔	✔	D	✔	D	✔		✔	
Eppendorf	*epMotion 5075t*	✔	✔	✔	✔	✔	✔	✔			

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

Target Enrichment

KAPA HyperCap Probes for hybridization-based target enrichment

Better by Design

Combining more than a decade of probe-design experience with an improved manufacturing process, **KAPA HyperChoice Probes** (for human designs) and **KAPA HyperExplore Probes** (for non-human designs) are fully customizable target enrichment panels for hybridization-based capture before next-generation sequencing. Answer your most challenging research questions with probes manufactured using KAPA HiFi DNA polymerase and validated by NGS.

Easily design customized probe pools with our HyperDesign software, place your order for KAPA HyperExplore or KAPA HyperChoice probes, and then combine the probes with our KAPA HyperPrep or KAPA HyperPlus Library Preparation Kits to:

- **Reduce sequencing costs** with superior capture uniformity
- **Reliably enrich challenging, previously inaccessible genomic regions**
- **Streamline target enrichment** using the updated KAPA HyperCap Workflow, driven by KAPA HyperPrep or KAPA HyperPlus Library Preparation Kits

Reduce sequencing costs with superior capture uniformity

Achieve better coverage, higher uniformity, and low PCR duplication rates (**Table 3, Figure 4**) with high-fidelity KAPA HyperCap probe panels designed using the expertise of HyperDesign (**HyperDesign.com**).

Table 3. New KAPA HyperCap designs yield low PCR duplication rates, high uniformity, and broad target coverage

Design	Genes	Target size (capture)	HQ reads	% total duplicates	Fold-80 (uniformity)	% of bases ≥30X
Neurodegenerative	>98	335 Kb	1.75 M	2.78	1.38	99.83
Hereditary disease	>4100	12.3 Mb	20.00 M	1.62	1.43	97.22

Target-enriched libraries were prepared from 100 ng of Coriell NA12891 DNA following the KAPA HyperCap Workflow v3 with KAPA HyperPrep Kit

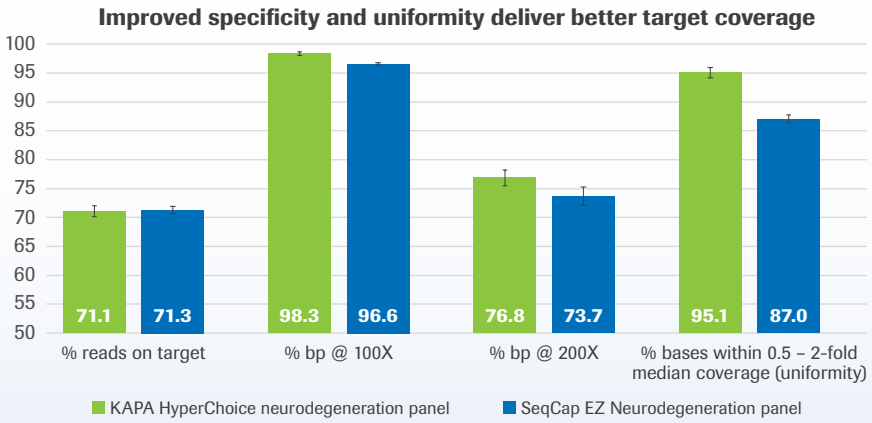


Figure 4. The new KAPA HyperChoice neurodegeneration probe panel outperforms the SeqCap EZ Neurodegeneration panel with higher uniformity and better target coverage, without the need for rebalancing. Performance was compared between the SeqCap EZ Neurodegeneration panel and a new KAPA HyperChoice panel covering similar regions. For each panel, 8 target-enriched DNA libraries were prepared from Coriell control DNA using the appropriate workflow: KAPA HyperCap v2 (SeqCap EZ) or KAPA HyperCap v3 (KAPA HyperChoice). Libraries were captured in 8-plex reactions and sequenced on an Illumina MiSeq instrument (2 x 100 bp). The KAPA HyperChoice panel was used out-of-the-box, while the SeqCap EZ panel was empirically rebalanced to improve performance.

Target Enrichment

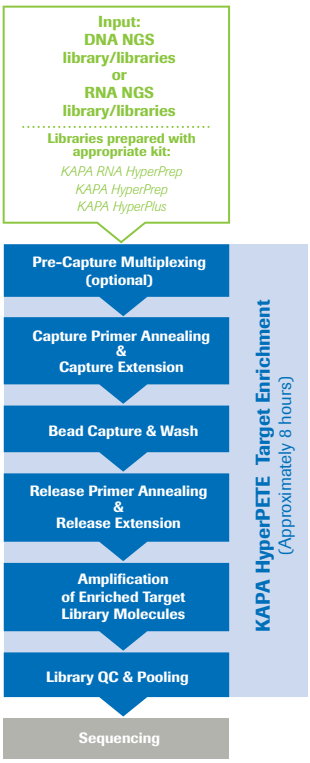
KAPA HyperPETE Primers for Primer Extension Target Enrichment

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 FFPE-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, automatable workflow**
- **Achieve superior performance and coverage uniformity**
- **Uncover critical genomic information from a wide variety of sample types, including FFPET and cfDNA**
- **Reliably enrich challenging, previously inaccessible genomic regions**



Save valuable time with an efficient, single-day, automatable workflow

- Take hours off of total workflow time compared to typical hybridization capture, with time requirements similar to amplicon and anchored multiplex methods (**Figure 5**)
- Enrich for long contiguous regions, using fewer tubes per sample compared to amplicon technologies

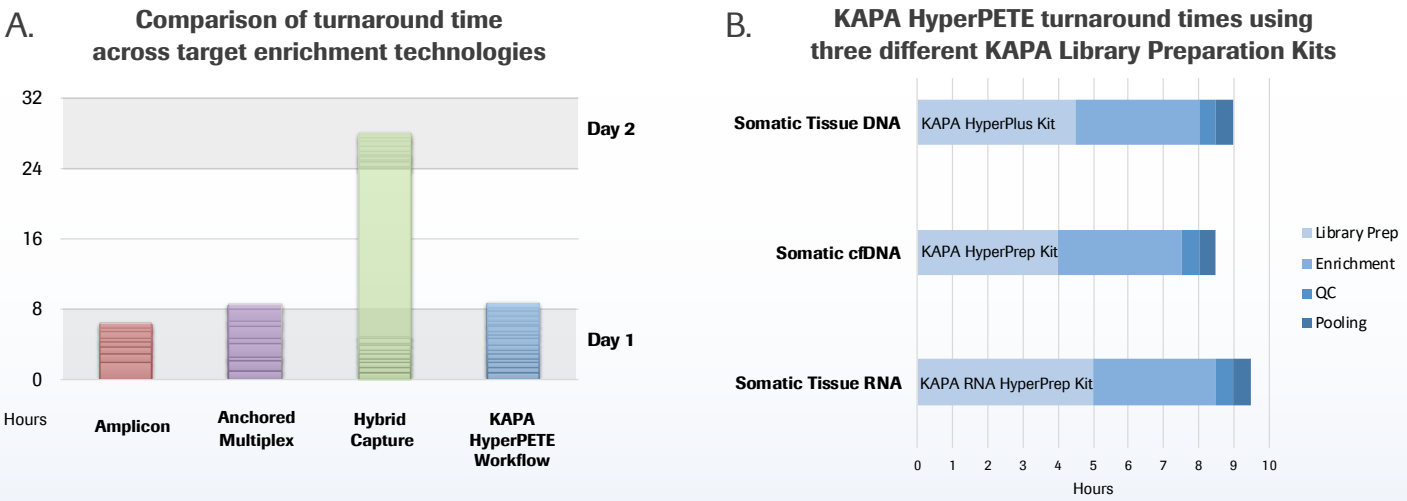


Figure 5. The turnaround time (TAT) for KAPA HyperPETE target enrichment is similar to the TAT for amplicon-based workflows. (A) While most hybridization-based workflows take two days to complete, KAPA HyperPETE workflows can be completed in one day. **(B)** Differences in the TAT for various applications 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.

Primer Extension Target Enrichment

A new way of enriching DNA or RNA targets

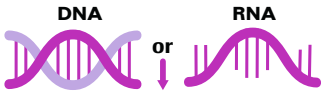
PETE is a novel NGS hybridization capture technology *designed to employ primer extension reactions to specifically capture and release target library molecules for sequencing.*

Superior performance and coverage uniformity

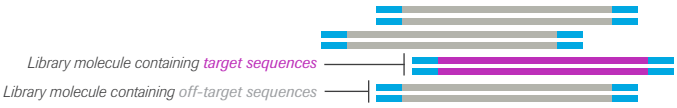
Fewer manual steps

Faster workflow

Start with DNA or RNA



1. **Prepare indexed libraries:** Prepare libraries with KAPA DNA or RNA Library Prep Kits and truncated, universal adapters (▬) —with or without UMIs.



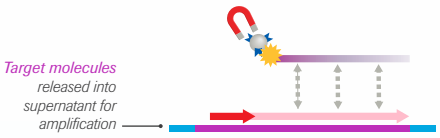
2. **Anneal target-specific capture primers:** Heat-denature libraries and hybridize to biotinylated target-specific capture primers (✸➡); for simplicity, only one strand of each denatured library molecule is shown.

3. **Perform capture primer extension:** Library molecules containing target sequences will form biotin-labeled capture-ready extension products, while off-target library molecules will not.

4. **Capture and wash target library molecules:** Use paramagnetic streptavidin beads (✸) and a magnet (∩) to capture and immobilize target molecules, and then wash away off-target molecules. The remaining library will be greatly enriched for target sequences.

5. **Anneal target-specific release primers:** Hybridize captured library molecules to target-specific release primers (➡); the binding sites for these primers are upstream of the capture primer sites.

6. **Perform release primer extension:** Primer extension releases the target molecules into the supernatant to be collected for amplification; the biotin-labeled molecules remain behind on immobilized beads.



7. **Amplify target library molecules:** Use universal library amplification primers (➡) to amplify the released, target-enriched library molecules, and then perform cleanup.

Finish with a sequencing-ready, target-enriched library

For more information, please visit: *go.roche.com/KAPAHyperPETE*

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

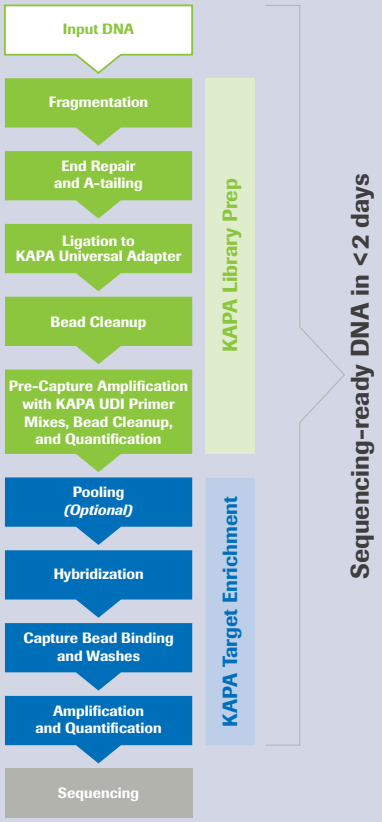
...then let our advanced algorithm do the work. Once probe selection is complete, you'll be able review the coverage results across your target regions.

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 without the need for a SpeedVac™**—now with more automation friendly volumes, and moderate hybridization and wash temperatures (55ºC and room temp)



Library Quantification

KAPA Library Quant and Roche LightCycler®

Why is qPCR-based library quantification preferred for library QC?

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.

KAPA Library Quantification Kits contain all reagents needed for qPCR-based quantification of NGS libraries for Illumina® sequencing.

- Accurate quantification of sequencing-competent libraries (**Figure 6**)
- Better accuracy when pooling libraries
- Automation-friendly workflow for increased throughput

Roche LightCycler® 96 and LightCycler® 480 Instruments** ensure reproducible, reliable, accurate data.

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

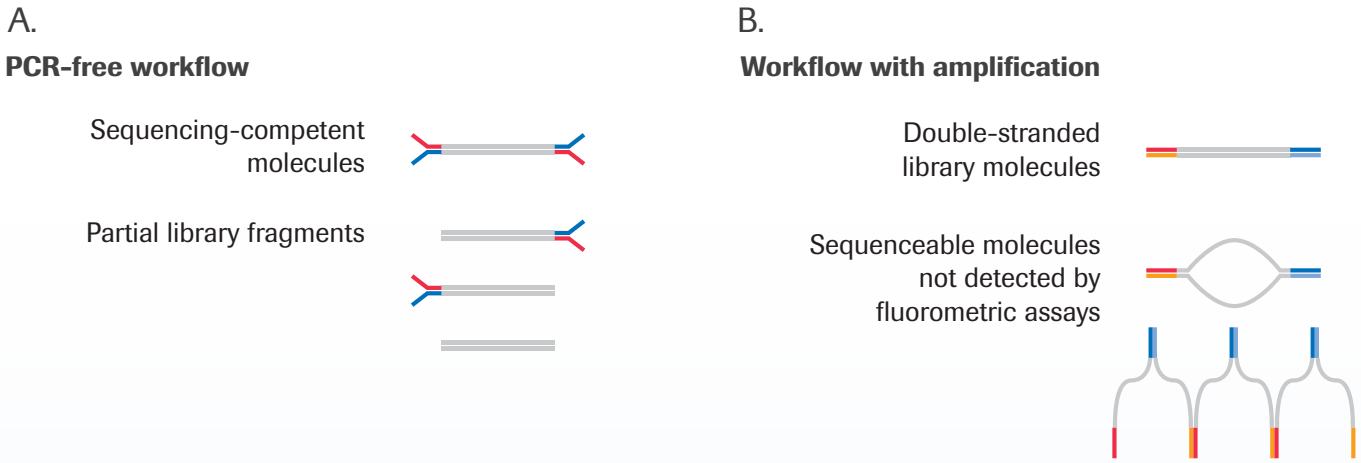


Figure 6. Library quantification via qPCR-based methods, such as the KAPA Library Quantification Kit, enables accurate sample pooling and optimal clustering.

(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.

Ordering Information

	Roche cat. no.	KAPA code	Description	Kit size	HyperCap Workflow	HyperPETE Workflow
Nucleic Acid Purification	07290519001		MagNA Pure® 24 System	1 Instrument		
	06541089001		MagNA Pure 96 System	1 Instrument		
	09189823001		KAPA NGS DNA Extraction Kit	24 rxns		x
	09190023001		KAPA NGS DNA Extraction Kit	96 rxns		x
	09217215001		KAPA NGS FFPE DNA Polishing Kit	24 rxns		x
	09217223001		KAPA NGS FFPE DNA Polishing Kit	96 rxns		x
Sample Quantification/QC	09217193001		KAPA NGS FFPE DNA QC Kit	24 rxn		x
Library Prep	07962312001	KK8500	KAPA HyperPrep Kit	8 rxns	x	x
	07962347001	KK8502	KAPA HyperPrep Kit	24 rxns	x	x
	07962363001	KK8504	KAPA HyperPrep Kit	96 rxns	x	x
	07962380001	KK8510	KAPA HyperPlus Kit	8 rxns	x	x
	07962401001	KK8512	KAPA HyperPlus Kit	24 rxns	x	x
	07962428001	KK8514	KAPA HyperPlus Kit	96 rxns	x	x
	08963835001	KK8007	KAPA HyperPure Beads	5 mL	x	x
	08963843001	KK8008	KAPA HyperPure Beads	30 mL	x	x
	08963851001	KK8009	KAPA HyperPure Beads	60mL	x	x
	08963860001	KK8010	KAPA HyperPure Beads	450 mL	x	x
Adapters and Primers	09063781001		KAPA Universal Adapter, 15 µM	960 µL	x	x
	09063790001		KAPA Universal Adapter, 15 µM	4 x 960 µL	x	x
	09134336001		KAPA Unique-Dual Indexed (UDI) Primer Mix 1-96	96 rxns	x	x
	09329838001		KAPA Unique-Dual Indexed (UDI) Primer Mix, 97-192	96 rxns	x	x
	09329846001		KAPA Unique-Dual Indexed (UDI) Primer Mix, 193-288	96 rxns	x	x
	09329854001		KAPA Unique-Dual Indexed (UDI) Primer Mix, 289-384	96 rxns	x	x
KAPA HyperCap Enrichment Reagents	09075810001		KAPA HyperCapture Reagent Kit	24 rxns	x	
	09075828001		KAPA HyperCapture Reagent Kit	96 rxns	x	
	09075917001		KAPA HyperCapture Reagent Kit	4 x 96 rxns	x	
	09075879001		KAPA Probes Resuspension Buffer	1 mL	x	
	09075887001		KAPA Probes Resuspension Buffer	2 mL	x	
	09075763001		KAPA Hybrid-Enhancer Reagent	1 mL	x	
	09075836001		Roche Universal Enhancing Oligos	24 rxns	x	
	09075852001		Roche Universal Enhancing Oligos	96 rxns	x	
	09075895001		Roche Universal Enhancing Oligos	4 x 96 rxns	x	
KAPA HyperPETE Enrichment Reagents	09211624001		KAPA HyperPETE Reagent Kit	24 rxns		x
	09211683001		KAPA HyperPETE Reagent Kit	96 rxns		x
Beads	09075780001		KAPA HyperCapture Bead Kit	24 rxns	x	x
	09075798001		KAPA HyperCapture Bead Kit	96 rxns	x	x
	09075909001		KAPA HyperCapture Bead Kit	4 x 96 rxns	x	x
KAPA HyperCap Probes *(Inquire for larger packs)*			KAPA HyperChoice for custom human designs	12 to 10,000 rxns	x	
			KAPA HyperExplore for custom non-human designs	12 to 10,000 rxns	x	
			KAPA HyperCap Heredity Panel	12 to 192 rxns	x	
			KAPA HyperCap Oncology Panel	12 to 48 rxns	x	
KAPA HyperPETE Primers *(Inquire for larger packs)*			KAPA HyperPETE Choice and Explore for custom human designs	96 to 1536 rxns		x
			KAPA HyperPETE Pan Cancer Panel	24 to 384 rxns		x
			KAPA HyperPETE Hereditary Oncology Panel	24 to 384 rxns		x
			KAPA HyperPETE Hot Spot Panel	24 to 384 rxns		x
Real-Time PCR for Library QC	05815916001		LightCycler® 96	1 Instrument	x	x
	05015278001		LightCycler® 480 (96-well)	1 Instrument	x	x
	05015243001		LightCycler® 480 (384-well)	1 Instrument	x	x
	07960298001	KK4854	KAPA Library Quantification Kit for Illumina® Platforms: LC480 qPCR Master Mix	500 rxns	x	x
	07960140001	KK4824	KAPA Library Quantification Kit for Illumina Platforms: Universal Master Mix	500 rxns	x	x

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sequencing.roche.com

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

*All MagNA Pure 24 and MagNA Pure 96 Kits, Consumables, and Accessories are for *in vitro* diagnostic use.
**For Life Science Research Use Only. Not for use in diagnostic procedures.
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