rhAmpSeq TARGET AMPLICON SEQUENCING SYSTEM

NGS power. PCR simplicity.

Obtain high multiplexing levels by minimizing primer-dimer formation and misprimed PCR products

Perform only 2 PCR amplification steps with a fast, easy workflow

Achieve accurate targeted sequencing results with high on-target rates and uniform coverage

The rhAmpSeq system enables highly accurate amplicon sequencing on Illumina® next generation sequencing (NGS) platforms. The result: easy-to-use amplicon sequencing that is both high-performance and cost-effective.

rhAmpSeq technology has numerous research applications, including:

- Agricultural biotechnology
- CRISPR genome editing analysis
- Human disease research
- Human sample tracking and analysis

This system couples our proprietary RNase H2-based PCR technology, rhAmp PCR, with a streamlined workflow that generates NGS-ready amplicon libraries in just 2 amplification steps (Figure 1).

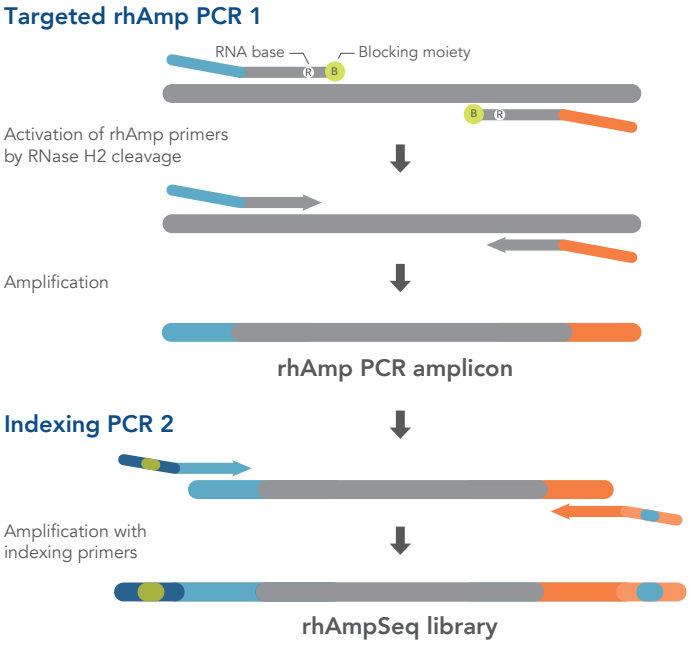


Figure 1. Overview of amplification steps in the rhAmpSeq workflow.

HARNESSING THE POWER OF rhAmp PCR

rhAmp PCR technology increases the specificity of PCR amplification while minimizing the biggest limitation of PCR multiplexing: primer-dimer formation.

RNase H2 activates rhAmp primers by target-specific cleavage of the RNA base within the DNA:RNA duplex, removing the 3′ blocker (Figure 1). RNase H2 activity is highly specific, thus reducing the amount of non-specific hybridization and primer-dimers. Only activated primers can be extended to generate target amplicons.

Illumina sample indexes and P5/P7 sequences are incorporated during the second, universal amplification step. Dual-indexed rhAmpSeq libraries are ready to be pooled and sequenced using Illumina sequencing instruments.

SIMPLE WORKFLOW, FEW COMPONENTS

The rhAmpSeq system saves both time and reagent costs with its simple workflow. Generate NGS-ready amplicon libraries in just 2 PCR amplification steps with either a custom or predesigned panel, library kit, and index primers (Figure 2).

Figure 2. Overview of the rhAmpSeq system workflow and components.

RHAMPSEQ LIBRARY PREPARATION MATCHES YOUR THROUGHPUT NEEDS

The rhAmpSeq system can be used with either a regular or a high-throughput protocol. Both protocols are automation friendly. The regular protocol offers the highest uniformity of coverage across samples, while the high-throughput protocol reduces both overall workflow time and cost by removing cleanup steps and the need to quantify and normalize DNA libraries before pooling (Table 1).

Table 1. Select the best rhAmpSeq library preparation protocol for your needs.

Performance and workflow considerations	Regular protocol	High-throughput protocol
Better sample-to-sample coverage uniformity	✓	
Better performance with challenging sample types (e.g., FFPE, cfDNA)	✓	
Ideal for high-throughput screening labs		✓
No library quantification and normalization required		✓
Hands-on time*	2.5–4.5 hr	1–1.5 hr
Total workflow time*	4–6 hr	4–4.5 hr

* Estimated time to process 12–96 samples using manual pipetting, including setting up reactions, cleanup, library quantification, and normalization steps

A VERSATILE TOOL

rhAmpSeq technology enables you to design large, custom panels with flexible amplicon sizes that can be tailored for specific applications, and to use as little as 10 ng of input DNA (Table 2). These capabilities make the rhAmpSeq system the ideal tool for high-throughput screening, sensitive analyses of challenging samples, and every targeted sequencing project in between.

Visit **www.idtdna.com/rhAmpSeqDesignTool** to start your custom design request.

Table 2. rhAmpSeq system features and specifications

Feature	Specification
Supported protocols	Regular library preparation (10–100 ng) High-throughput library preparation (10–50 ng)
Sample type	Tissue, FFPE, cfDNA
Insert size	Flexible (50–200 nt)
Custom panel size	Up to 5000 amplicons per panel
Sample indexing capability	96 index sequences (up to 9216 combinations)
Compatible platforms	Illumina

HIGH QUALITY PERFORMANCE

The rhAmpSeq system is optimized for consistent performance across panel sizes ranging from tens of amplicons to thousands of amplicons in a single multiplex reaction. Figure 3 shows representative performance of custom panels designed across a range of human hotspot targets. A slight performance boost can be observed with the regular library preparation protocol compared to the high-throughput protocol.

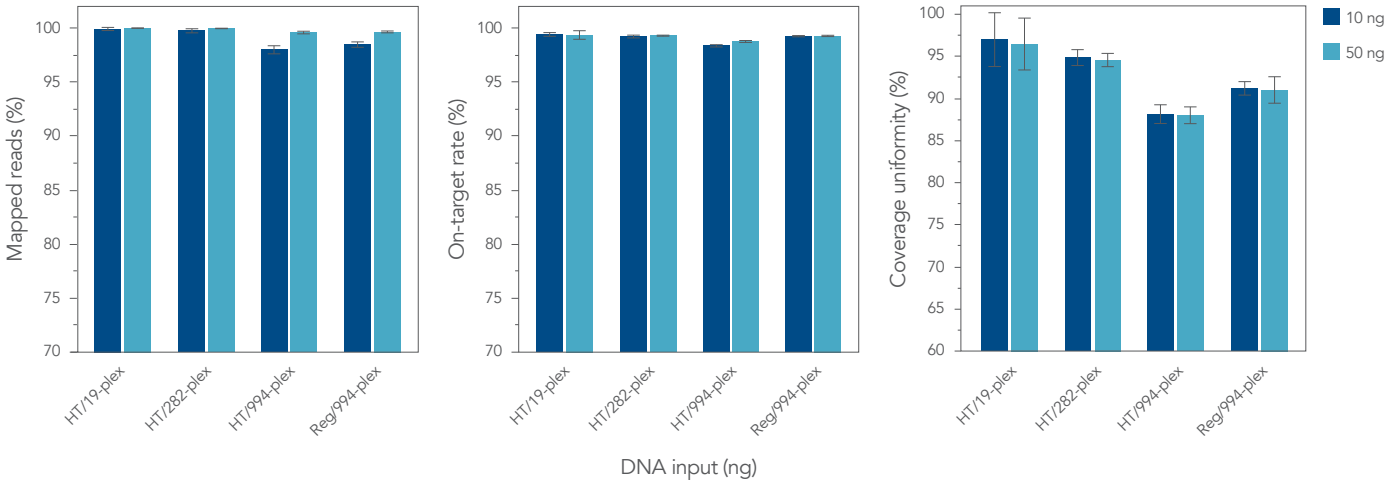
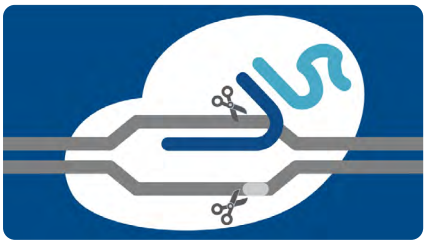


Figure 3. High-quality sequencing data across a range of non-optimized panel sizes and DNA input quantities. Coriell DNA samples were used to evaluate the performance of non-optimized rhAmpSeq panels of varying sizes (20, 282, and 994 amplicons) following the high-throughput (HT) protocol using 10 and 50 ng of DNA input. The largest panel (994 amplicons) was evaluated following the regular (Reg) protocol with 10 and 100 ng of DNA input. Coverage uniformity is the % of targets with coverage ≥0.2X of the mean. Error bars = standard deviation from the mean

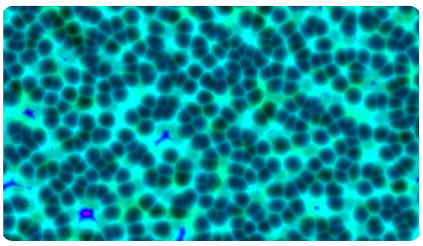
ONE SYSTEM, MANY APPLICATIONS

The rhAmpSeq system has a wide variety of research applications, and should be considered for any application that requires stringent and efficient sequencing analysis. These are just a few of the fields in which rhAmpSeq technology has been successfully used.

Genotyping by sequencing is an agricultural biotechnology technique for molecular breeding and trait/marker selection. Whether you are focused on a few markers for marker-assisted selection or on thousands of markers for genomic selection, the rhAmpSeq system can help accelerate your plant or animal improvement programs.



CRISPR genome editing has recently become a dominant technology. However, CRISPR editing can produce unwanted off-target editing events. Custom rhAmpSeq Panels save time and resources by allowing you to quickly interrogate many CRISPR-edited sites simultaneously, so you won't have to do single PCRs.



Detecting disease-associated variants is a key element of human-focused research. With the rhAmpSeq system's combined specificity and multiplexing capabilities, you can easily and accurately detect "hotspot" variants. In addition, the rhAmpSeq Sample ID Panel can be used to track samples and avoid mix-ups in human sample workflows.

To start your Custom rhAmpSeq Panel design, visit www.idtdna.com/rhAmpSeqDesignTool.

ORDERING INFORMATION

Product	Size	Catalog #
Custom rhAmpSeq Panel (pool or plate)	0.4 nmol	Request custom design
	4 nmol	
	8 nmol	
rhAmpSeq Sample ID Panel	16 rxn	10000082
	96 rxn	10000083
rhAmpSeq Library Kit	16 rxn	10000064
	100 rxn	10000065
	500 rxn	10000066
	5000 rxn	10000067
rhAmpSeq Index Primer (i5 or i7)	6 nmol	Choose sequences when ordering

> For more information, visit **www.idtdna.com/rhAmpSeq**

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