rhAmpSeq™ CRISPR library preparation

For targeted amplicon sequencing

For Research Use Only. Not for use in diagnostic procedures. | Version 2

REVISION HISTORY

Version	Release date	Description of changes
2	January 2023	Updated template.
1	January 2021	Initital release.

Table of contents

INTRODUCTION

This protocol provides instructions for the preparation of next generation sequencing (NGS) libraries with rhAmpSeq CRISPR targeted sequencing reagents. These libraries are amplified from genomic DNA samples that have either been edited by CRISPR enzymes or are unedited, control DNA samples that are being compared to CRISPR-edited DNA samples. This version of the protocol describes a high-throughput workflow resulting in targeted libraries from your DNA samples that are ready for sequencing on Illumina® platforms.

Advantages of rhAmpSeq CRISPR amplicons

rhAmpSeq CRISPR amplicons can be used to quantitate the degree of editing at numerous on-target CRISPR sites, therefore they are most useful for assessing efficiency. When considering on-target assessment, the sites intended to be targeted by the CRISPR nuclease define the design space. In addition to efficiency, rhAmpSeq CRISPR amplicon panels also enable assessment of off-target activity. So, the target design space for rhAmpSeq CRISPR panels includes not only the known on-target locus but also, very importantly, any number of empirically-defined off-target sites. The off-target sites must be experimentally determined with predictive methods such as GUIDE-Seq, or other methods of empirical nomination. You are able to supplement the empirically-defined off-target sites with *in silico* predictions derived from tools, such as the IDT **CRISPR-Cas9 guide RNA design checker**.

Advantages of using this protocol

This protocol describes the NGS library preparation that precedes CRISPR analysis. Perform this streamlined procedure with Custom rhAmpSeq CRISPR Panels that includes two PCR steps to create NGS-ready, paired-end libraries for short-read Illumina sequencing.

This protocol eliminates bead clean-up after the Targeted rhAmp PCR 1 step and uses efficient clean-up, quantification, and normalization of libraries procedures. After generating libraries for NGS, you can proceed to CRISPR analysis with our cloud-hosted analysis tool that assists you in understanding the data.

OVERVIEW

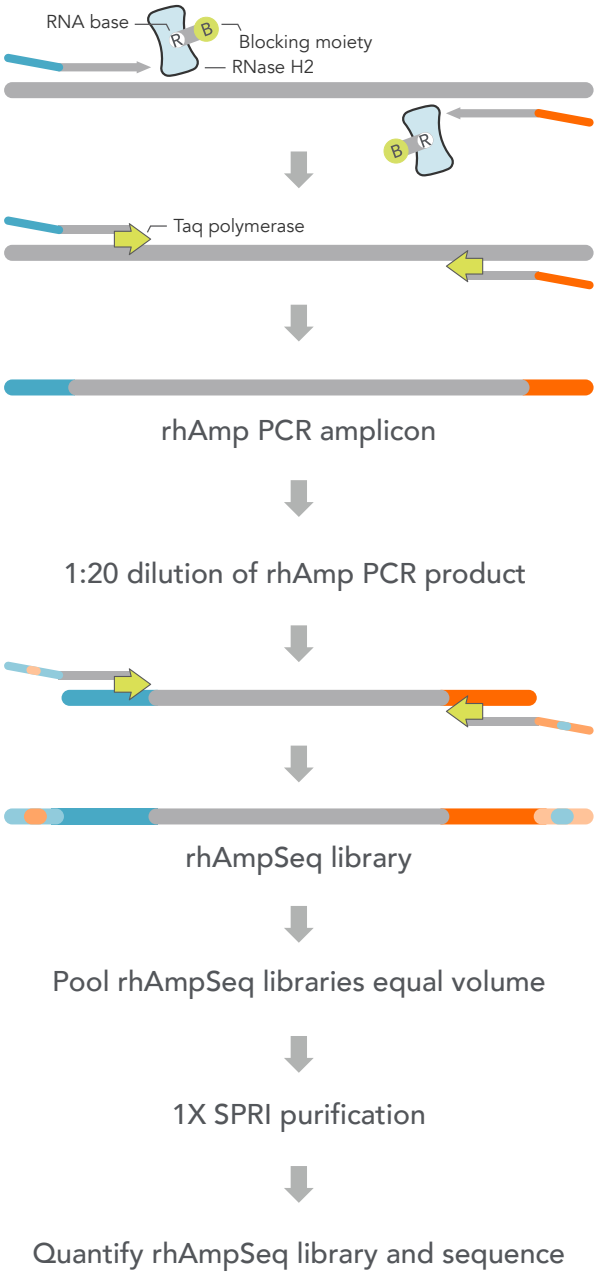
Amplicon sequencing is a highly targeted approach that enables you to analyze CRISPR editing events at specified genomic regions representing on- and off-target editing sites. The rhAmpSeq CRISPR Analysis System is comprised of an innovative suite of reagents for targeted amplicon sequencing based on proprietary IDT rhAmp PCR chemistry. This novel solution enables highly accurate sequence analysis for thousands of targets with a fast and simple workflow.

The rhAmpSeq CRISPR workflow involves 2 PCR amplifications that generate sequence-ready libraries (Figure 1). In the first PCR step, combine Custom rhAmpSeq CRISPR Panels with rhAmpSeq CRISPR Library Mix 1 to amplify regions of interest. In the second PCR step, add rhAmpSeq Index Primers and Library Mix 2 to append sample indexes and P5/P7 sequences to the PCR 1 amplicon, completing the library building process.

Targeted rhAmp PCR 1

Activation of rhAmp primers by RNase H2 cleavage

Amplification by Taq polymerase



Indexing PCR 2

Amplification of rhAmp PCR amplicons with i7 & i5 index primers

Figure 1. Overview of the 2-step PCR high-throughput rhAmpSeq workflow.

rhAmpSeq CRISPR ANALYSIS SYSTEM COMPONENTS

Panels

Your custom rhAmpSeq CRISPR Panel contains rhAmp primers for targeted amplification in your species of interest. For more information on panel design, visit www.idtdna.com/rhAmpSeqCRISPRpanels.

Library kit

The rhAmpSeq CRISPR Library Kit includes 2 PCR master mixes (Library Mix 1 and Library Mix 2) specifically formulated for use with rhAmpSeq CRISPR panels. The 4X library mixes contain the required enzymes and buffer components necessary for library construction. Purchase of the rhAmpSeq CRISPR Library Kit includes access to Analysis Credits to enable data processing and quantification of edits using IDT's rhAmpSeq CRISPR Analysis Tool.

Tip: Your unique analysis credits code is automatically sent to you in email shortly upon order fulfillment. Your available balance of analysis credits displays when you login to the tool.

Index primers

rhAmpSeq Index Primers allow for incorporation of unique sample indexes, as well as the addition of P5 and P7 adapter sequences. The use of sample indexes allows for multiplexing of samples during sequencing to increase throughput.

GLOSSARY

Term	Definition
-plex	Refers to the plexity of the panel or the number of assays in the panel, each of which target a different region within the gDNA. For example, a single pair of rhAmp Primers (FWD/REV) will result in a 1-plex assay, while a 96-well plate of primers (1 plate with FWD, 1 plate with REV) will result in a 96-plex panel.
IDTE buffer	An IDT-proprietary buffer that contains 10 mM Tris and, importantly, 0.1 mM EDTA at either pH 7.5 or pH 8.0. Use of IDTE buffer is critical to the success of PCR in the rhAmpSeq CRISPR workflow.
Primer dimer	By-products of PCR. They occur when primers bind to each other, instead of the intended target DNA, and elongation occurs. This reaction results in unintended amplicons.
rhAmpSeq CRISPR assay	An IDT-proprietary assay that consists of Forward (FWD) and Reverse (REV) rhAmp Primers designed to amplify a specific DNA region.
rhAmpSeq CRISPR panel	A collection of rhAmpSeq CRISPR assays. The rhAmpSeq CRISPR Forward Pool contains all the Forward rhAmp Primers, while the rhAmpSeq CRISPR Reverse Pool contains all the Reverse rhAmp Primers.
rhAmp primer	A specific type of primer used in a rhAmpSeq CRISPR assay (e.g., Targeted rhAmp PCR 1 in the rhAmpSeq CRISPR workflow). These primers contain a ribobase with a 3′ blocker and are designed to be unblocked by a thermophilic RNase H during PCR.

CONSUMABLES AND EQUIPMENT

Consumables—IDT

Reagent	Size	Catalog #	Storage temperature
IDTE, pH 7.5	10 x 2 mL	11-01-02-02	Room temperature
IDTE, pH 8.0	10 x 2 mL	11-01-02-05	
Nuclease-Free Water	10 x 2 mL	11-04-02-01	
rhAmpSeq CRISPR Library Kit* (Comprised of Library Mix 1 and 2)	16 rxn/credits 100 rxn/credits 500 rxn/credits 5000 rxn/credits	10007317 10007318 10007319 10007320	–20°C
rhAmpSeq CRISPR Panel (Comprised of FWD and REV panels)	Varies	Varies	
rhAmpSeq Index Primer (i5)	6 nmol	Varies	
rhAmpSeq Index Primer (i7)	6 nmol	Varies	

*Each kit size includes the indicated CRISPR analysis credits to apply towards data analysis.

Consumables—Other suppliers

Consumable	Supplier	Catalog #
Agencourt® AMPure® XP- PCR Purification beads	Beckman Coulter	A63880 – 5 mL A63881 – 60 mL A63882 – 450 mL
Axygen® 8-Strip PCR Tubes, 0.2 mL	Thermo Fisher Scientific	14-222-251
DNA LoBind Tubes, 1.5 mL	Eppendorf	022431021
Eppendorf twin.tec® microbiology PCR Plate 96, semi-skirted		0030129326
Microseal® 'B' PCR Plate Sealing Film	Bio-Rad	MSB1001
Ethanol (200 proof)	General supplier	Varies
Qubit™ dsDNA High Sensitivity Assay Kit, or Qubit BR Assay Kit (or equivalent)	Thermo Fisher Scientific	Q32851 Q32850
Qubit assay tubes	Thermo Fisher Scientific	Q32856

Equipment

Equipment	Supplier	Catalog #
96-well thermal cyclers	General laboratory supplier	Varies
Microcentrifuge		
Vortex mixer		
DynaMag™-2 Magnet, or equivalent	Alpaqua	A000400
Qubit Fluorometer, or equivalent	Thermo Fisher Scientific	12321D
Centrifuge with plate adapter	General laboratory supplier	Varies

GUIDELINES

Sample input DNA

The high-throughput rhAmpSeq CRISPR library preparation protocol is optimized for 10–50 ng of purified genomic DNA input. In general, the more input DNA, the better performance you can expect within the recommended range (10–50 ng). See Appendix: Troubleshooting for more information.

Tip: Store rhAmpSeq CRISPR reagents (rhAmpSeq CRISPR Panels, Library Mixes, and Index Primers) at –20°C and avoid multiple freeze-thaw cycles.

Important: Perform AMPure XP bead clean up on the same day as PCR amplification.

Workflow

1. Prepare reagents	• Make a 10X rhAmp Primer Pool • Prepare rhAmpSeq Index Primers	Total time: Varies
2. Perform Targeted rhAmp PCR1	• Set up Targeted rhAmp PCR 1 • Run the PCR 1 Program	Total time: ~3 hr
3. Dilute PCR 1 product	• Make a 1:20 dilution of the rhAmpSeq PCR 1 product	Total time: ~5 min
4. Perform Indexing PCR	• Set up Indexing PCR 2 • Run the PCR 2 program	Total time: ~50 min
5. Pool libraries	• Prepare the rhAmpSeq CRISPR library pool	Total time: Varies
6. Clean up library	• Purify the indexed rhAmpSeq CRISPR library	Total time: ~30 min

PROTOCOL

Prepare reagents

Make a 10X rhAmp Primer Pool

IDT supplies rhAmp primers in one of two formats:

- Pooled and ready-to-use (Go to **Prepare the rhAmpSeq Index Primers** section to begin)

or

- Individually plated (dried down)

1. If you have individual (dried down) rhAmp primers, use the following formula to determine the amount of IDTE, pH 7.5 needed to resuspend each to a final concentration of 50 µM (0.05 nmol/µL):

$$\text{Amount of IDTE, pH 7.5} = \frac{\text{nmol of rhAmp primer}}{0.05\ \text{nmol/µL}}$$

For example:

$$\text{Amount of IDTE, pH 7.5} = \frac{0.4\ \text{nmol of rhAmp primer}}{0.05\ \text{nmol/µL}} = 8\ \text{µL}$$

Important: Use IDTE, pH 7.5 (10 mM Tris; 0.1 mM EDTA, pH 7.5), or equivalent. Do not resuspend in water or TE buffer.

2. Centrifuge all rhAmp primer plates before resuspension.
3. Resuspend each rhAmp primer to 50 µM using the volume of IDTE determined in **Step 1**.
4. Seal the plates, vortex to fully resuspend, then briefly centrifuge.
5. Combine equal volumes of each individual 50 µM stock rhAmp primer to create stock forward or reverse primer pools.

Note: Forward primers are combined to make a forward rhAmp Primer Pool, and reverse primers are combined to make a reverse rhAmp Primer Pool.

Important: Do not combine forward and reverse rhAmp primers until you are ready to perform the Targeted rhAmp PCR 1.

6. Create a 10X rhAmp Primer Pool for your rhAmpSeq CRISPR assay. Use this table to determine the appropriate 10X concentration based on the plexity of your primer pool.

Panel size	10X rhAmp primer concentration	Calculate 10X rhAmp Primer Pool working concentration
≥500-plex	50 µM (total)	50 µM (FWD and REV pool)
101-plex < X <499 plex	100 nM (each primer)	100 nM × # of primers (FWD or REV pool)
≤100-plex	250 nM (each primer)	250 nM × # of primers (FWD or REV pool)

7. If necessary, dilute the 50 µM forward or reverse rhAmp Primer Pool to the appropriate 10X working concentration using IDTE, pH 7.5.

   Important: If the rhAmp Primer Pool needs to be diluted, use IDTE, pH 7.5 (10 mM Tris; 0.1 mM EDTA, pH 7.5), or equivalent.

8. Store the stock rhAmp primer plates and forward and reverse rhAmp Primer Pools at –20°C.

   Important: Do not combine forward and reverse rhAmp primers for long-term storage.

Prepare the rhAmpSeq Index Primers

rhAmpSeq Index Primers (i5 or i7) are supplied as individual dried down primers (6 nmol) and need to be resuspended.

1. Resuspend the rhAmpSeq Index Primer to 100 µM by adding 60 µL of IDTE, pH 8.0. Vortex to resuspend, then centrifuge.

   Important: Use IDTE, pH 8.0 (10 mM Tris; 0.1 mM EDTA, pH 8.0), or equivalent. Do not resuspend in water or TE buffer.

2. Dilute rhAmpSeq Index Primer to 1 µM using IDTE, pH 8.0.
3. Make aliquots to minimize the number of freeze-thaws.
4. Store the dilutions at –20°C.

Perform Targeted rhAmp PCR 1

Set up Targeted rhAmp PCR 1

Before performing Targeted rhAmp PCR 1, you must completely thaw the rhAmpSeq CRISPR forward and reverse pools and 4X rhAmpSeq Library Mix 1 to room temperature (15–20°C).

Note: This protocol demonstrates using 96-well PCR plates; however, strip tubes can be used instead.

1. After thawing the following reagents, briefly vortex, then centrifuge:
   - 10X rhAmp PCR Panel—Forward Pool
   - 10X rhAmp PCR Panel—Reverse Pool
   - 4X rhAmpSeq CRISPR Library Mix 1
2. Dilute gDNA to 0.91 ng/µL—4.55 ng/µL using IDTE, pH 8.0.
3. Add 11 µL of diluted gDNA to each reaction well in a 96-well plate (10–50 ng total input).

   Tip: For quantification of DNA, use a Qubit dsDNA Quantitation Assay Kit, or equivalent.

   Important: Not all DNA quantitation methods produce equivalent results.

4. Add the following reagents to each reaction well containing diluted gDNA:

Reagent	Volume (per reaction)
4X rhAmpSeq CRISPR Library Mix 1	5 µL
10X rhAmp PCR Panel—Forward Pool	2 µL
10X rhAmp PCR Panel—Reverse Pool	2 µL
Total volume, including 11 µL of diluted gDNA sample	20 µL

5. Seal the Targeted rhAmp PCR 1 plate, then briefly vortex and centrifuge.

Run the Targeted rhAmp PCR 1 program

1. Place the Targeted rhAmp PCR 1 plate in a thermal cycler and run the Targeted rhAmp PCR 1 thermal cycler program as described, with a heated lid set to 105°C:

Targeted rhAmp PCR 1 program			
Step	Cycle	Temperature (°C)	Duration
Activate enzyme	1	95	10 min
Amplify	14	95	15 sec
		61	8 min
Deactivate enzyme	1	99.5	15 min
Hold	1	4	∞ Hold

2. Upon removing the Targeted rhAmp PCR 1 plate from the thermal cycler when the program completes, **proceed *immediately*** to Dilute PCR 1 product.

Dilute PCR 1 product

Make a 1:20 dilution of the rhAmp PCR 1 product

1. Briefly vortex the PCR 1 product, then centrifuge.
2. Add 95 µL of Nuclease-Free Water to each reaction well of a new 96-well plate.
3. Transfer 5 µL of the PCR 1 product to the 95 µL Nuclease-Free Water (1:20 dilution).
4. Seal the plate, thoroughly vortex to mix, and centrifuge before you **proceed *immediately*** to Perform Indexing PCR 2.

Perform Indexing PCR 2

Set up Indexing PCR 2

Use room temperature 4X rhAmpSeq CRISPR Library Mix 2 and Index Primers i5 and i7 for this section. At this time, remove the Agencourt AMPure XP beads from refrigerated storage and bring to room temperature.

Note: Use a different combination of i5 and i7 index primers for each sample that is combined in a multiplex sequencing run.

Tip: Using the rhAmpSeq primers as UDIs (unique dual indexes) is expressly recommended for off-target editing analysis.

1. Briefly vortex, then centrifuge the 4X rhAmpSeq CRISPR Library Mix 2 and Index Primers i5 and i7.
2. Prepare Indexing PCR 2 in a new 96-well plate, as shown:

Reagent	Volume (per reaction)
4X rhAmpSeq CRISPR Library Mix 2	5 µL
Indexing PCR Primer i5 (1 µM)	2 µL
Indexing PCR Primer i7 (1 µM)	2 µL
rhAmpSeq CRISPR PCR 1—1:20 dilution	11 µL
Total volume	**20 µL**

3. Seal the indexing PCR 2 plate, then briefly vortex and centrifuge.

Run the PCR 2 program

1. Place the Indexing PCR 2 plate in a thermal cycler, then run the Indexing PCR 2 thermal cycler program as described, with a heated lid set to 105°C:

rhAmpSeq Indexing PCR 2 program			
Step	Cycle	Temperature (°C)	Duration
Activate enzyme	1	95	3 min
Amplify	24	95	15 sec
		60	30 sec
		72	30 sec
Final extension	1	72	1 min
Hold	1	4	∞ Hold

2. Remove the Indexing PCR 2 plate from the thermal cycler after the program completes and proceed immediately to Pool libraries.

Pool libraries

Prepare the rhAmpSeq CRISPR library pool

Make the library pool by combining equal volumes of all your indexed libraries.

1. With a multichannel pipette, transfer 5 µL of each indexed library into an 8- or 12-well strip tube (use 10 µL if the total number of libraries is less than 20).

Tip: Minimize sample-to-sample variability with precise pipetting.

2. Cap the strip tube, briefly vortex, then quickly centrifuge.
3. Combine all libraries from the strip tube into a single 1.5 mL reaction tube.

Important: Pool the complete volume from each of the wells of the 8-well strip tube for even sample coverage.

4. Store the remaining individual indexed libraries at –20°C, then proceed immediately to Clean up library.

Clean up library

Purify the rhAmpSeq CRISPR library

1. With the Agencourt AMPure XP beads at room temperature, vortex thoroughly before use.
2. Prepare an 80% ethanol solution by combining 1 part molecular-grade water and 4 parts molecular-grade ethanol (200 proof).

Important: Use fresh 80% ethanol to avoid a loss in assay performance.

3. Transfer 100 µL of the rhAmpSeq CRISPR library pool to a new 1.5 mL tube.
4. Add 100 µL of AMPure XP beads (1X) to the library pool.
5. Thoroughly pipette mix the contents of the tube.
6. Incubate for 10 minutes at room temperature.
7. Briefly centrifuge the tube, then place it on a DynaMag™-2 Magnet, or equivalent, for 5 minutes, or until the solution is clear.

8. Keeping the tube on the magnet, do the following:
   a. Aspirate, then discard the supernatant.
   b. Add 1000 µL of 80% ethanol to the tube.
   c. Incubate at room temperature for 30 seconds.
   d. Aspirate, then discard the supernatant.
   e. Repeat the 80% ethanol wash one more time for a total of 2 washes (Steps 8b–d).
   f. Use a fresh pipette tip to remove all traces of ethanol from the tube.
   g. Allow the beads to dry for 3 minutes at room temperature.
9. Remove the 1.5 mL tube from the magnet.
10. Add 22 µL of IDTE, pH 8.0, to elute rhAmpSeq CRISPR library pool.
11. Thoroughly vortex to fully resuspend the beads, then briefly centrifuge the tube.
12. Incubate at room temperature for 3 minutes.
13. Place the 1.5 mL tube on the magnet to collect the beads for 1 minute, or until the solution is clear.
14. Keeping the 1.5 mL tube on the magnet, transfer 20 µL of the final rhAmpSeq CRISPR library pool elution into a new 1.5 mL tube, ensuring no beads are carried over.
15. Proceed to quantify, then sequence your rhAmpSeq CRISPR library pool.
    Follow the instructions for your specific Illumina platform.
16. Store any remaining library pool at –20°C for up to 3 weeks.

SEQUENCING

To perform NGS sequencing on your rhAmpSeq CRISPR libraries, follow the instructions for your specific Illumina sequencer.

Following sequencing, you can use the rhAmpSeq CRISPR sequencing analysis credits that are included in the kit purchase to log in to our cloud-hosted analysis platform, **rhAmpSeq CRISPR Analysis Tool**, and upload FASTQ files and panel-specific .bed files to process pair-end read data via CRISPRAltRations.

Refer to the rhAmpSeq CRISPR Analysis Tool **video tutorial** for help in navigating the analysis tool.

APPENDIX

Troubleshooting

For more information on our rhAmpSeq CRISPR system, go to our FAQ web page.

Issue	Possible cause	Action
Low percent of sequence reads map to the genome	High percentage of primer dimers	Determine if the unmapped reads are due to primer dimers by aligning the sequence reads to the primer sequences.
	Contaminating DNA	Determine if the unmapped reads are due to DNA contamination by performing a BLAST search and aligning to different genomes.
High percent of primer dimers	Low amount of DNA input	• Requantify the DNA to determine if the concentration is correct. • Add more DNA input to the reaction.
	Forward and reverse primers were stored together in a pool	Forward and reverse primers should not be pooled together; they should be stored as separate forward and reverse pools.
	The primers in the 10X pool are at the wrong concentration	Ensure that the correct primer concentration is used when making the 10X forward and reverse pools.
	Cross-reaction between primers from different assays	Although rare, primers from different assays can amplify to form primer dimers. If possible, remove one or both of the assays from your panel.
	AMPure purification after PCR 2 was not performed according to the protocol	Ensure that the correct ratio of AMPure beads to sample is used.
Within a panel, the total reads between samples are variable (greater than a two fold difference)	Not enough PCR 2 cycles	With larger panels (>1000-plex), the number of cycles in PCR 2 may need to be increased so that all of the reactions reach saturation.
	The sample DNA input was outside the recommended range of 10–50 ng	Requantify the DNA input to ensure that the sample DNA input is between 10 and 50 ng.
	Indexing PCR primer is not at correct concentration	Check that the rhAmpSeq Index Primers (i5 and i7) were at the correct concentration when they were diluted before PCR 1. (See **Prepare the rhAmpSeq Index Primers**). The rhAmpSeq Index Primers are limiting reagents in the **Indexing PCR 2 reaction** and are used to normalize the library concentration between samples. Differences in the rhAmpSeq Index Primer concentrations will cause different samples to have more or fewer total reads than other samples.

Issue	Possible cause	Action
Some assays have less coverage than others.	Less efficient assay	The coverage of individual assays can be increased by increasing the forward and reverse primer concentrations of those assays. When adjusting primer concentrations, make sure the final forward and reverse pools still follow the recommended guideline for pool creation.
	Improper formulation of the Forward and/or Reverse Panel	Check that the rhAmpSeq CRISPR Forward and Reverse Panels were formulated correctly.
	Allelic bias	Allelic bias due to primer design uses a reference genome. If a relatively high-frequency SNP is present in the test DNA but is not shared by the reference genome, the primer will have a difficult time annealing. rhAmpSeq primers are designed with N-bases at positions that might overlap a SNP to enable primer annealing. Unexpected heterozygous variants affecting primer annealing may cause the amplicons from that chromosome to be under-represented in the sequencing data. Unexpected homozygous variants affecting primer annealing may fully prevent amplification of the genomic target. Test this possibility by amplifying genomic DNA from sources unlikely to contain the same variant.
Little to no library yield generated	Not enough DNA input	• Requantify the DNA to double-check the concentration and/or measure the DNA integrity. • Increase DNA input in the reaction.
	Protocol was not followed at every step	Deviations from the protocol, including using the wrong reagents, not setting up the PCR reactions correctly, or not completely sealing the PCR plates can negatively affect the performance of the assay.
	TE buffer was used instead of IDTE to resuspend and/or dilute the rhAmpSeq primers and/or the Indexing PCR Primers	Make sure to use IDTE instead of TE buffer. Standard TE buffer contains more EDTA than IDTE and can inhibit the PCR reactions.
	AMPure purification was not performed according to the protocol	Follow the protocol as written and make sure that the 80% ethanol is made fresh on the day it is needed.

Target drop-out	Analytical pipeline filtered on-target reads	Reads mapping to each target will contain a unique primer pair. If the pipeline discarded those reads incorrectly, they will persist in the unmapped read files. To find them, search the FASTQ files for primer sequences associated with low-coverage targets. If you find reads containing the expected primer sequences at frequencies higher than the target coverage, it could be that your pipeline incorrectly discarded those reads.
	In rare cases, a primer pair may not amplify the target due to an unknown SNP occurring in the genome	(See **Allelic bias**, above) Sequence the genomic region underlying the primer using Sanger sequencing. **Contact us** to redesign the affected primer.
	Primer concentrations were too low	Quantitate the assay primers using a NanoDrop™ spectrophotometer (Thermo Fischer Scientific).
	The primer-targeted area does not exist, or exists differently, in your target genome	• Check that the region can be amplified in single-plex. • Check if the genomic area containing your target of interest can be amplified. Submit the amplicon for Sanger sequencing to verify it contains the expected genomic sequence.
	FLASH discards target amplicons smaller than read length	By default, FLASH (used for R1/R2 merging) will discard reads that are longer than the length of the read. This can be overcome by using the -O option in the command line call to Flash. The -O command tells Flash to accept outliers. See here for more information.

rhAmpSeq™ CRISPR library preparation

For more information, go to: **www.idtdna.com/ContactUs**

> For more than 30 years, IDT's innovative tools and solutions for genomics applications have been driving advances that inspire scientists to dream big and achieve their next breakthroughs. IDT develops, manufactures, and markets nucleic acid products that support the life sciences industry in the areas of academic and commercial research, agriculture, medical diagnostics, and pharmaceutical development. We have a global reach with personalized customer service.

Doc ID: RUO22-1386_001 01/23