Notices

Limitations of use

For research use only. Not for use in diagnostic procedures. Unless otherwise agreed to in writing, IDT does not intend these products to be used in clinical applications and does not warrant their fitness or suitability for any clinical diagnostic use. Purchaser is solely responsible for all decisions regarding the use of these products and any associated regulatory or legal obligations.

Safety data sheets pertaining to this product are available upon request.

Safety notices

Reminder symbols call attention to minor details that may be easily overlooked and compromise the procedure resulting in decreased performance.

Caution symbols denote critical steps in the procedure where risk of protocol failure or damage to the product itself could occur if not carefully observed.

Revision history

Version	Release date	Description of changes
RA-DOC-668 R01	October 2026	Updated document ID from legacy system (RUO22-1454_001). Updated genome list based on recent analysis tool updates. Corrected formatting throughout.

Contents

Revision history 2
Background 4
Introduction 8
Access the tool 8
Create new designs 8
Appendix A: Input requirements and troubleshooting 18
Troubleshooting 21
Access terms and conditions 25

Technical support

Visit https://www.idtdna.com/pages/support for a helpful answers to frequently asked questions or contact us directly at applicationsupport@idtdna.com.

Background

rhAmp PCR technology enables precise target amplification

The rhAmpSeq CRISPR Analysis System enables highly accurate amplicon sequencing on Illumina® next generation sequencing (NGS) platforms. This system leverages RNase H-dependent PCR (rhAmp PCR), a novel technology developed at IDT, to improve target specificity and reduce both off-target amplification and primer-dimer formation. rhAmp primers contain a 3' blocking modification and a single RNA base. When rhAmp primers anneal to their specific target, a thermostable RNase H2 enzyme cleaves the RNA base, activating the primer and allowing extension to occur (**Figure 1**).

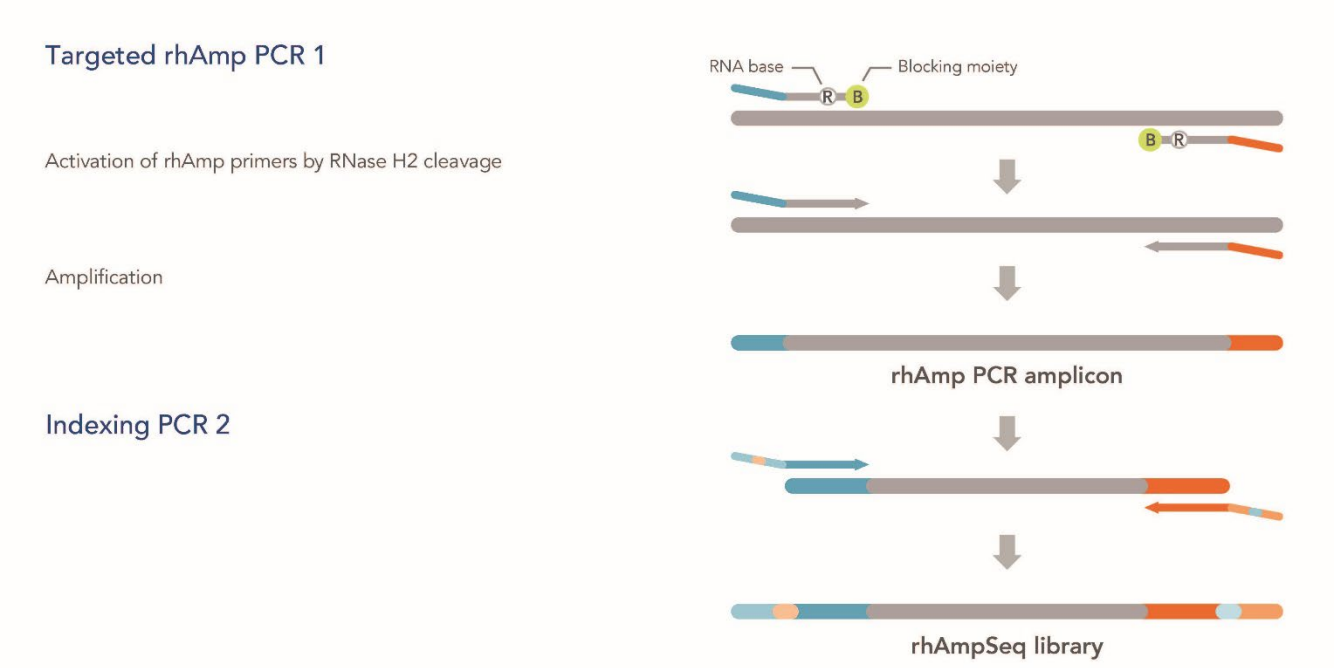


Figure 1: Amplification steps in the rhAmpSeq workflow. RNase H2 activates rhAmp primers by target-specific cleavage of the RNA base within the DNA:RNA duplex, removing a 3' blocker. RNase H2 activity is limited to correctly paired DNA:RNA duplexes, thus reducing the amount of amplification from non-specific hybridization and primer dimers. Only activated rhAmp primers can be extended to generate target amplicons. Illumina sample indexes and P5/P7 sequences are incorporated during the second amplification step.

In the rhAmpSeq method, PCR amplification is carried out on a highly multiplexed scale, allowing for precise amplification of up to 5000 targets in a single reaction. Samples that are processed through the rhAmpSeq workflow are ready to be sequenced on any Illumina platform (**Figure 2**).



Figure 2: Overview of the rhAmpSeq CRISPR Analysis System workflow. rhAmpSeq CRISPR Panels consist of rhAmp primer pairs designed to minimize primer dimers and maximize multiplexing capability for high quality amplicon libraries. They are designed for uniform coverage and along with efficient library prep, allows you to confidently analyze more samples, faster.

rhAmpSeq design process overview

rhAmpSeq panel design is a two-step process that provides performance and workflow advantages over other amplicon sequencing systems:

1. **Assay design** involves extensive target site review and rhAmp primer design for each target, followed by comprehensive QC of each primer to mitigate off-target effects, such as non-specific genomic hybridization.
2. **Virtual assay pooling** is a unique multiplex primer QC feature that further enhances the specificity of rhAmp PCR technology. It allows us to more accurately predict potential primer-dimer amplification products that could lower overall reaction efficiency and decrease the percentage of correctly mapped reads.

During the assay design and virtual pooling process the design pipeline maximizes the multiplexing and performance of a single pool. Due to sequence composition, some assays may be placed in a secondary pool.

When there is a secondary pool, the design tool automatically creates secondary panels (indicated by a “P2” prefix in the panel name). After attempting another secondary pooling step, some assays may still not be poolable. These assays will go into the singles collection (indicated by an “SC” prefix in the panel name).

Note: The assays in the singles collection are meant to be amplified individually in library prep reactions since they are not compatible with other assays in the panel. Considering this, the assays in the singles collection can only be ordered in plate format, and not as a pre-formulated pool of assays.

After the rhAmpSeq CRISPR workflow is completed, libraries made from secondary pools and singles collections can be combined with the main pool and sequenced together on the same sequencing run.

Glossary

Term	Definition
Assay ID	A unique ID generated by the rhAmpSeq Design Tool for each target in a panel. The Design Detail report will show the original Target name and associated Assay ID for each target (see section Use the Design Tool Dashboard for more information about the Design Detail summary).
BED coordinate	A text file format used to define genomic regions by coordinates in a chromosome or FASTA sequence.
cfDNA	Cell-free DNA is found circulating in the bloodstream without encapsulation in a cell. cfDNA is often very short (<150).
Design run ID	The unique ID generated by the rhAmpSeq Design Tool. Design run IDs can be used to search for panels in the rhAmpSeq Design Tool Dashboard.
FASTA sequence	A text-based format showing a nucleotide (or protein) sequence.
FFPE DNA	DNA from formalin-fixed, paraffin-embedded (FFPE) tissue. FFPE DNA is often degraded and can be difficult to amplify.
Hotspot	A single base or short genomic region that is likely to be mutated.
Insert size	The number of bases between the forward and reverse primers.
Panel name	A user-defined name associated with each panel.
Primary pool	A collection of rhAmpSeq assays. The rhAmpSeq Forward Pool contains all the Forward rhAmp Primers, while the rhAmpSeq Reverse Pool contains all the Reverse rhAmp Primers.
Required targets	A list of targets that should be prioritized for adding to the primary pool. Required targets should be a subset of the full target list.
rhAmp primers	A specific type of primer used in rhAmp SNP assay or rhAmpSeq assay. These primers contain a ribobase with a 3' blocker and are designed to be unblocked by a thermophilic RNase H during PCR.
Secondary pool	A collection of rhAmpSeq assays that are not compatible with some assays in the primary pool. The secondary pool will also have a pool of forward primers and pool of reverse primers. The panel name will have a "P2." prefix, while the Design run ID will have a ".P2" suffix.

Singles collection	A collection of rhAmpSeq assays that were not compatible with the primary pool nor secondary pool. Assays in the singles collection must be processed as individual PCR reactions. The panel name will have an "SC." prefix, while the Design run ID will have a ".SC" suffix. (When ordering assays from the singles collection, product labels and associated documentation designate these as "non-pooled" assays).
Target name	A user-defined name associated with a specific target in a panel.

Introduction

The rhAmpSeq Design Tool can assist you in ordering your unique panels and empower you to have those designs in hand quickly. Once your design is ready, order your panels and begin your research. The panels you design can also be queried, viewed, or shared at any time.

Access the tool

Open the rhAmpSeq Design Tool at www.idtdna.com/rhAmpSeqDesignTool.

Log into your IDT web account with your user name and password to access the tool.

Create new designs

In the rhAmpSeq Design Tool, choose your application-specific selections in the Create new design window.

Application

Select either:

- Hotspot genotyping (SNPs and short indels)
- CRISPR gene editing analysis (indels)
- **Note:** If you don't see your application listed, contact us.

Input format

Select either:

- Coordinates (BED)
- FASTA sequence
- **Note:** For more information see Data input formats in Appendix A.

Species

The rhAmpSeq Design Tool uses reference genomes to perform QC on the designed primers, ensuring high specificity.

Select from one of the following species profiles:

Reference genomes
Bos taurus - Cow (ARS UCD1.2)
Bos taurus - Cow (ARS UMD3.1)
Caenorhabditis elegans - round worm (WBcel235)
Canis lupus familiaris – Dog (CanFam 3.1)
Danio rerio - zebrafish (GRCZ11)
Gallus gallus - Chicken (GRCg6a)
Gossypium hirsutum - Cotton (ASM98774v1)
Homo sapiens-Human (GRCh37)
Homo sapiens-human (GRCh38)
Macaca fascicularis - crab-eating macaque (macFas5)
Macaca fascicularis - crab-eating macaque (MFA1912RKSv2)
Macaca mulatta - rhesus monkey (rheMac8)
Mus musculus - Mouse (mm39)
Mus musculus-mouse (GRCm38)
Oryza sativa - Rice (IRGSP1.0)
Rattus norvegicus - rat (Rnor6)
Sus scrofa - Pig (11.1)
Vitis riparia – Grape (EGFV_Vit_rip_1.0)

Note: If you want to submit a set of targets for a species that is not found in the menu, select Other. When making this selection, you will be asked to submit FASTA sequences and realize that no genome QC will be performed.

Note: If you have a reference genome for your target species that is not listed, submit a custom design request here.

Insert size

Insert size refers to the number of base pairs between the rhAmpSeq forward and reverse primers. IDT has determined the optimum default minimum and maximum insert sizes for rhAmpSeq assays depending on whether you are running a Hotspot or CRISPR application (see the table below). However, these values can be changed based on your sequencing criteria and project goals.

Application	Default minimum insert size	Default maximum insert size
Hotspot	70 bp	120 bp
CRISPR	120 bp	200 bp

IDT recommends selecting a minimum insert size of at least 50 bp. We do not have a recommendation for maximum insert size because this may vary depending on your project goals. However, our R&D team has observed generally lower coverage for longer amplicons due to either the poor cluster amplification efficiency of longer amplicons on Illumina instruments, or the preference for shorter amplicons to be covered, compared to longer amplicons. Generally, a smaller range between minimum and maximum insert size results in better amplicon coverage uniformity, whereas a bigger insert range gives the design pipeline more flexibility in designing primers at the target site and may increase overall panel design success.

Note: For degraded DNA samples, such as FFPE or cfDNA, you will most likely need to have a shorter insert size, e.g. 50–100 bp.

Important! Make sure that your maximum insert size does not exceed the read length of your sequencing kit. For example, if you plan to use a 300–cycle kit, do not allow your maximum insert size to exceed 300 bp.

Panel name

Provide a name for your panel design. This name will identify your panel and appear on all relevant documentation, as well as on tubes and plates in your order. A maximum of 12 characters will be used for product labels containing your panel name.

Note: The name you provide secondary pools and singles collections will be appended with a prefix or suffix to help indicate it as such.

Provide your target inputs

Enter your target input by entering manually or copy/pasting the FASTA data into the space provided. You can also upload a file in the Upload file tab.



Optional – Advanced parameters

Requires targets

If there are any targets that must be included in the primary pool, copy and paste the target names into the field provided. For BED inputs, these are the coordinates of the targets. For FASTA sequences, these are the names identifying each target sequence. These targets are a subset of the targets in the main input field.

Important! The rhAmpSeq Design Tool will prioritize these targets over others in your panel.

Make compatible with pre-existing Custom rhAmpSeq Panel

If you plan to add content to a pre-existing Custom rhAmpSeq Panel, or if you want to combine this panel with another predesigned Custom rhAmpSeq Panel, enter the Design run ID of the pre-existing Custom rhAmpSeq Panel in the field provided.

Note: If any of your new target designs conflict with pre-existing assays, the new designs will be excluded from the primary pool.

Make compatible with rhAmpSeq Sample ID Panel

The rhAmpSeq Sample ID Panel is a predesigned panel containing 76 assays used to track and manage human samples in NGS workflows.

Select Yes if you want to combine your sample panel with the IDT rhAmpSeq Sample ID Panel.

Note: If any of your new target designs conflict with the assays from the rhAmpSeq Sample ID Panel, the new designs will be excluded from the primary pool.

Balance the number of assays in each pool (if more than 1 pool is designed)

Select Yes for the balance option when more than 1 pool is designed for the target set.

Note: If all your assays are pooled into the primary pool, this feature would not apply.

As described in the rhAmpSeq design process overview section, the design pipeline will automatically create secondary (P2) panels if some of the assays were not able to be pooled into the primary panel. If you order these secondary panels, balance the number of assays in the primary and secondary panels, so that you do not need to normalize assay concentrations based on number of assays per pool when you combine libraries before sequencing.

Submit for design

Click “Design” to submit your request.

The rhAmpSeq Design Tool has a feature that checks your target inputs to maximize the design success rate of Custom rhAmpSeq Panel designs.

The input QC check should only take a few minutes to complete. If you do not have any input errors, you will see the following message, indicating that your inputs have been successfully submitted and your design is in process:

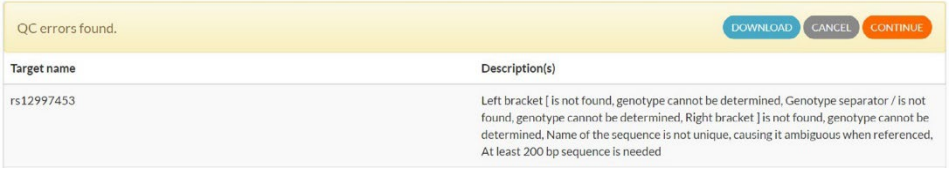


Once your panel designs are ready, you will receive an email. How quickly your design completes our QC pipeline depends on your panel size. Small panels may take <1 hour, while larger panels may take up to 2 days.

Note: We do not provide the sequences of the panels until your order has been placed.

Input QC check

The input QC check verifies correct formatting of your inputs, in addition to adherence to the rhAmpSeq pipeline design rules. If your target inputs do not process immediately upon clicking Design, you may see an error message like this:



Review the descriptions associated with each target.

You have the option to:

- Download an Excel file containing the list of your targets and the QC message associated with each target
- Cancel the design submission and fix the issues with the relevant targets, then resubmit the corrected inputs
- Continue with the design submission

Important! If we find errors with your target inputs during the QC check, the result could be a very low assay design success rate.

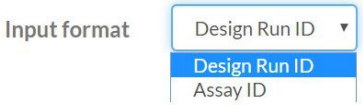
Note: Some of the issues identified during the input QC check may not need to be corrected. For example, some messages were meant to inform you that 2 targets in close proximity to each other were merged into a single target. In such cases, click “Continue” to submit the design.

Note: If you received an error message, check Appendix A: Input requirements to help resolve issues.

Review design results

To review your design results, go to www.idtdna.com/rhAmpSeqDesignTool, then click on the Review design results tab.

1. Select between format types:



2. Type either your Design run ID or your Assay ID into the space provided, then click Search.

Note: If the Design run ID is associated with multiple panels, you will receive the following message:

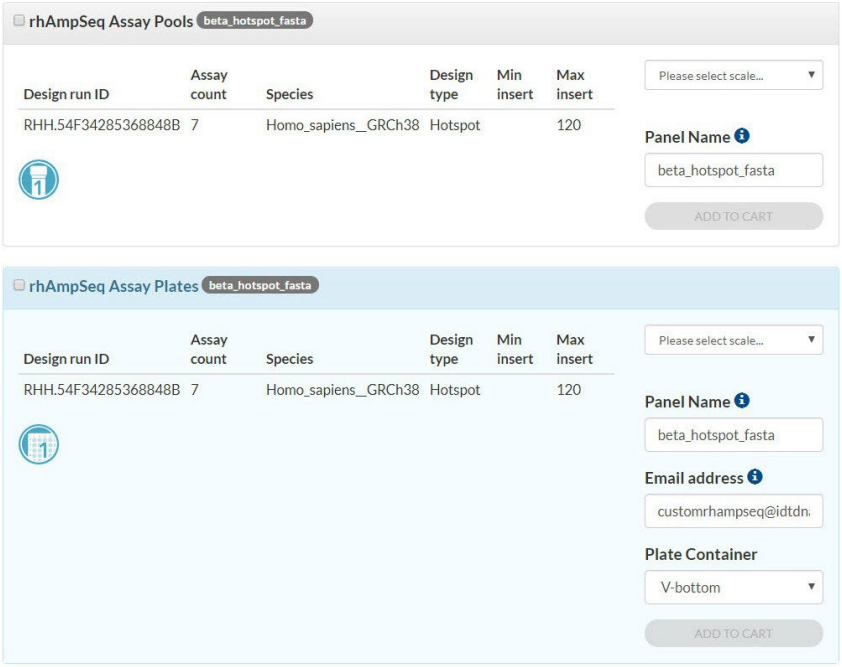
This Design run ID returned multiple pools. The Design Tool creates a Secondary Pool if any of the assays were not compatible with the Main Pool. Secondary Panels are designated with “P2.” at the beginning of their names. Assays that are not compatible with either primary or secondary pools are added to the singles collection, which provide assays in individual wells of a plate. The singles collection panel has “.SC” at the beginning of its name. For more information, see the rhAmpSeq Design Tool User Guide.

Your results will show a separate box for the primary, secondary, and singles collection panels, as available. Each panel will be displayed as a pool of primers (white box) as well as a plate (blue box).

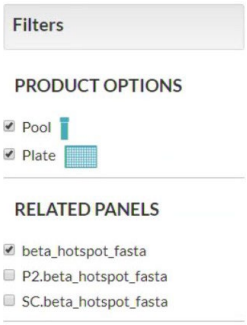
Note: Pools are delivered in a tube that is preformulated and ready to use, while plates deliver individual primers plated in each well. Orders delivered in plates will have primers normalized to the indicated scale and dried down.

Once you have located your design, you can obtain the following:

- Summary report—provides an overview of the panel design, including a quality rank for each assay design; # of assays in the panels, and size histograms for inserts, assays, and amplicons
- BED files associated with the panel design
- Sub-panel ordering capability—download into Excel to get assay IDs for assays from any of the design run panels to potentially order a subset of your assays as a separate panel



3. Once your search results are returned, filter by selecting from the different options under the Filter header.
    a. Product options: show panels in either pool format (all assays combined into a single tube) or plate format (each assay provided in a separate well of 96 well plate).
    b. Related panels: if the Design run ID is associated with multiple pools, you can select which pools are shown in the results.



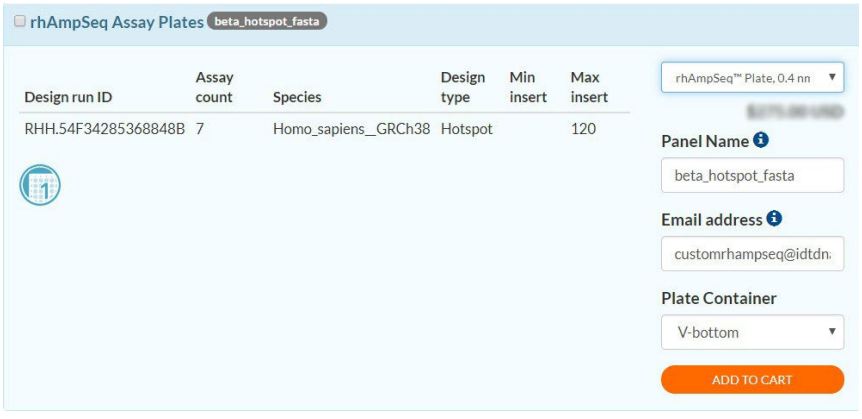
To place an order, select “scale” from the dropdown list, then click Add to cart.

Note: The panel name will appear as the prefix to any item loaded in the cart, and at least 2 items will automatically be loaded—FWD and REV. A maximum of 15 characters is used for the label.



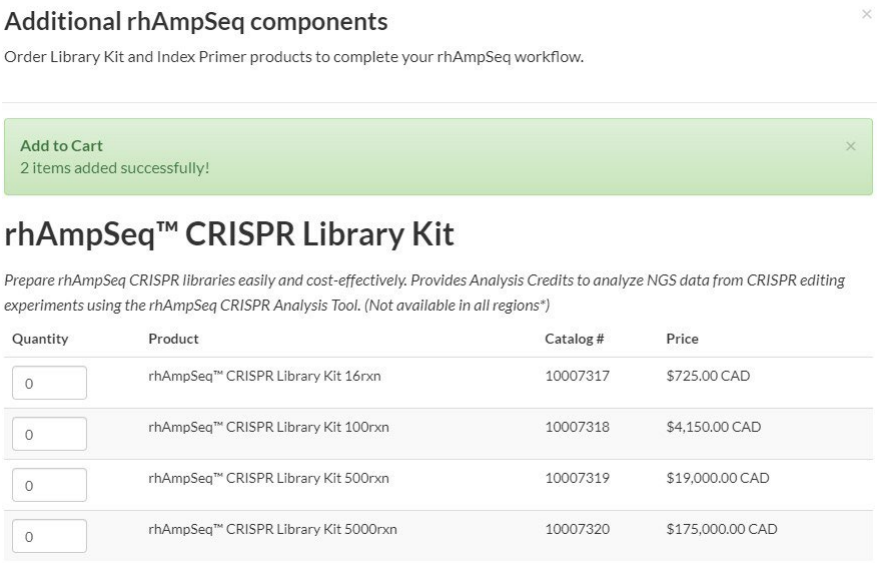
4. When ordering a plate, choose the type of plate.

Note: For the 0.4 nmol scale option, you must select V-bottom plates since the recommended resuspension volume is small and may cause resuspension and pipetting issues with larger plate formats.



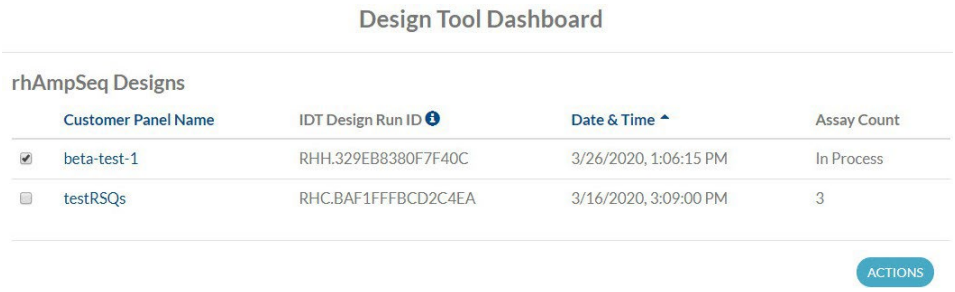
After adding an item to the cart, you will receive a pop-up request with the option to order other rhAmpSeq CRISPR components.

You will need to order a rhAmpSeq CRISPR Library Kit and rhAmpSeq Index Primers to complete the workflow.



Using the Design Tool Dashboard

To use the Dashboard, go to www.idtdna.com/rhAmpSeqDesignTool and click on the Design Tool Dashboard tab. All the panel designs you have created will be listed. If you would like to reorder a panel, click the link under Customer panel name. All panels associated with the Design run ID will appear in a list and can be ordered using process described in Review design results.



Select the checkbox next to the panel name, then click Actions to see the following options:

1. Download Excel—an Excel document with the target names and associated Assay IDs for every assay in the panel downloads. If applicable, the secondary pool and singles collection will also be available in separate tabs of the document.

Note: To order a subset of assays from your design, use this file to retrieve the Assay IDs.

2. Send to colleague—select this option to open a field for you to provide email recipients. Type in the email address of the person you would like to send this Design run ID, then click Send email. Your recipient will receive an automated email containing the rhAmpSeq Design run ID and be able to order this panel.

Available files

Other files available to download include:

- Excel file containing a list of target names and associated Assay IDs

Note: Use this file to order a panel containing a subset of the assays from the original panel.

- Design Summary Report
- BED files for analysis

Appendix A: Input requirements and troubleshooting

Sequence preprocessing: distance between targets and target size

Two parameters for rhAmpSeq target selection must be met before designing the rhAmpSeq panel. The first is the required minimum distance between targets—600 bp. The second is target size (sometimes termed “insert size” or “amplicon size”)—between 50 and 200 bp. Input data files will be preprocessed (i.e., include, exclude, or merge) to ensure workable target distance and size parameters.

Figure 2 illustrates an example of our preprocessing target analysis. In this example, Target_1 will be included in the rhAmpSeq design because it does not have any distance constraints and does not exceed the maximum target size.

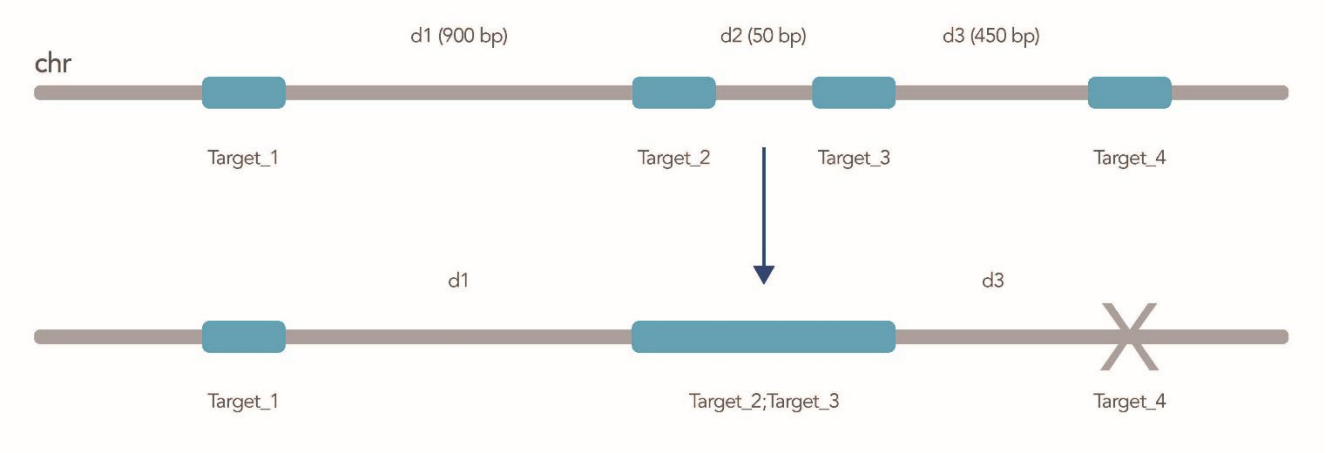


Figure 2: Preprocessing target analysis. The original targets (Targets 1–4) had different distances between them (d1 = 900 bp, d2 = 50 bp, and d3 = 450 bp). After preprocessing, the original 4 targets became 3 targets for rhAmpSeq design. Target 4 cannot be included in the primary panel design due to minimum distance requirement >600 bp between targets and its assay design will be placed in a secondary panel (or singles collection, if applicable).

Adjacent targets separated by less than 60 bp can be merged to generate a new, combined target. For example, Target_2 and Target_3 will be merged into a single, new target (named Target 2;Target 3). However, if the newly merged target size exceeds the maximum insert size of 200 bp, the targets will not be merged.

Finally, because Target_4 is 450 bp from Target_2;Target_3, it cannot be merged with Target2;Target3 (only targets that are <60 bp apart can be merged), and it cannot be included in the rhAmpSeq primary panel (distance between the targets is <600 bp). Any assay for Target_4 is therefore is placed in any applicable secondary panel or singles collection design.

Data input formats

BED file format

BED file for target of interest (not including flanking regions).

You can submit a BED file containing up to 6 columns, with columns 1–3 being required.

Note: Do not include a header in your BED file as the Design tool will consider this an incorrectly formatted target and will exclude it from the design.

1. Column 1 (required): **chrom**—the name of the chromosome (e.g., chr1, chr2)
2. Column 2 (required): **chromStart**—the starting position of the target in the chromosome
3. Column 3 (required): **chromEnd**—the ending position of the target in the chromosome. (chromEnd must be larger than the chromStart.)
4. Column 4: **name**—name for your target (While this is not a required field, we highly recommend providing unique IDs for each target in the file. If any targets share the same ID, the Design tool will assign a unique ID to each.)
5. Column 5: **Score**
6. Columns 6: **Strand** orientation (+ or –)

Example input:

```
chr11   34614227        34614228        rs10488741      0       +
chr11   87518023        87518024        rs7926017       0       +
chr11   88713708        88713709        rs6483391       0       +
chr11   124578234       124578235       rs76543769      0       +
chr12   18867007        18867008        rs10841100      0       +
...
```

FASTA file format

FASTA files should contain the name of the target (preceded by the “>” symbol), followed by the sequence of the target. Target SNPs should be denoted in bracket format with the reference sequence base first and the variant sequence base second (e.g., [A/T], where A is the reference base and T is the variant base. Where the mutation or SNP is unknown, simply indicate the location of mutation in square brackets using the wild-type allele (e.g., [A/A]). Additionally, the Design tool requires the sequence to include at minimum of 100 bp of sequence on either side of the target.

Example input:

```
>SNP_1
TCGGGGTTCACGAGCTTGCTCTCCTCTGCCATTCGCAATGGGCTGGCATAGGGCAGCCTCACTGCTTGGCTCCAGCCAGCGACTT
CAGGATGTGGCGATGAAGATGGGGTCTGGATGGGCGTTGGTATTCAGGATGTCGAGCCCCACTGGTCTGGGGGACCAGTTGGCGC
TTCCTGCAAAGGCATGTGCTCTGGGAAGGG
[A/T]
CTGGCCTGCTGCAGCAGCTCTGCAGAGGGGCCGGCCCCAGACTTGCCTATGTCATTAGTATAGCAGGTCCCGTTATTACCCGAGG
AGGCTTACAGGTTATCAGCGAGCTCCAGGAGCCactggagaaaggaagaagataaagaaggatttaaaaagaaaataacaaaaag
aaaaactgtattttctaATCCAAACCTTGC
>SNP_2
ATTGCCTTTCTGTGGAGCAAGGGGTGTTGTACACACAAGCCTCACTGTAGacactgcctcagtttccccataggCATAATGGGTC
CCTTCTAGTTCAGGCAATCTGGATTTGATCTTGAGTTCCAGTGCCAGCCTCTGGAGTCACTCCATTTTCATACCTTTTCATGATC
TCAGGGGCTCTGGGCAGTGGGAGGTGATGG
[C/G]
TTGGACAGATTCTTGGTCATGCTCCCCAACTCTTGGTGGCTCACCACTGAACACTCCAAACCCTGCTTAAAGAAGTTGATCTATC
TGAAAGCCAGGGTAAAGATTGCTAAGGCTTGTCTCCTCTCCCAGTGGGAAGAGAGAGGTTCTGTTGGTGTCCTGGTTGAATTGCT
TTGCAGAGAAGTCAATGCCCATCACCCTTG
...
```

Pooling constraints

Sometimes when you request a Custom rhAmpSeq Panel our design pipeline can design, but not pool some of your assays. This is due to the primer-dimer QC check during the pooling step of the process; any unpoolable assays would normally be discarded in the final primary panel of assays.

Note: If you want to receive these designable but unpoolable assays, you can access these secondary panels (designated “P2” in their panel name) in the Dashboard or under Related panels in the Review design results tab.

It is possible that some assays, even after attempting the secondary pooling step, are still not poolable. These assays would go into the singles collection (designated “SC” in their panel name). Access these panels in the Dashboard or under Related panels in the Review design results tab.

Note: The assays in the singles collection are designed to be amplified by themselves in library prep reactions, since they are not compatible with other assays in the panel. This means that the assays in the singles collection can only be ordered in plate format, and not as a preformulated pool of assays.

Troubleshooting

BED QC errors

Message	Suggested action	Notes
BED file input missing 1 or more fields. Chrom, chromStart, and chromEnd are required columns.	Check that the BED file contains the required fields. See Data input formats.	Design will be unable to proceed until this error is addressed.
chromStart coordinate must be smaller than the chromEnd coordinate.	Ensure that the value in column 3 is higher than the value in column 2.	
Chromosome or scaffold name is not found in the reference genome.	Check that the correct reference genome has been selected for the BED file.	
Duplicated target names have been altered to create a unique ID. New names will contain "..tx" where x = 2, 3, 4…	No action needed, unless you want to create your own unique IDs. If any targets have the same name, the Design Tool will append the name to make it unique. For example, if 3 targets are all names "SNP", the names will be changed to SNP, SNP..t2, and SNP..t3.	We recommend that you assign unique IDs to your target names. The Design report will only display unique IDs.
Target length exceeds the maximum insert size, please reduce target length or increase the maximum insert size in the tool settings.	Either increase the maximum insert size parameter or decrease the size of the target.	Design will be unable to proceed until this error is addressed.
Coordinate input exceeds chromosome length.	The coordinates referenced in the BED file fall outside the range of the chromosome found in the reference; modify the coordinates so they are within the chromosome.	

Flanking region is too small, must have 100 bp on either side of the target.	Modify the coordinates so they are not within 100 bp of the end of the target region.	
Target merged with another target, please see design summary for more details.	If two targets are close together, the Design Tool will attempt to merge them into a single target.	The design can proceed without intervention.
Distance between targets is too small, must be at least 600 bp. One target may be placed into a secondary pool.	If two targets are within 600 bp, the Design Tool may need to separate them into different pools.	
Merged targets exceed the maximum insert size. One target may be placed into a secondary pool.	You can increase the maximum insert size, but do not exceed the read length of your sequencing kit.	Merging of the two targets created a region that exceeded the maximum insert size.

FASTA QC errors

Message	Suggested action	Notes
Duplicated target headers have been altered to create a unique ID. New names will contain "..tx" where x = 2, 3, 4…	No action needed, unless you want to create your own unique IDs. If any targets have the same name, the Design Tool will append the name to make it unique. For example, if 3 targets are all named "SNP", the names will be changed to SNP, SNP..t2, and SNP..t3.	We recommend that you assign unique IDs to your target names. The Design report will only display unique names.
More than one instances of genotype notation found	Edit the FASTA sequence so that it contains on pair of brackets [ ] designating the target region. For sequence with multiple bracket pairs, expand the target regions so that a single pair of brackets encompasses all targets.	This indicates to the design pipeline to place primer sequences outside of the brackets and interrogate all target within.
Left bracket [is not found, genotype cannot be determined	No [found in the FASTA sequence, see Data input formats for more information.	Design will be unable to proceed until this error is addressed.
Right bracket] is not found, genotype cannot be determined	No] found in the FASTA sequence, see Data input formats for more information.	
Genotype separator / is not found, genotype cannot be determined	No / found in the FASTA sequence, see Data input formats for more information.	
Flanking region is too small, must have 100 bp on either side of the target	Expand the FASTA sequence so that there are at least 100 bases on either side of the genotype region.	
Genotype format is invalid	The genotype format was not recognized. Genotypes should be denoted in bracket format with the reference sequence base first and the variant sequence base second (e.g.,	

	[A/T], where A is the reference base and T is the variant base).	
Non IUPAC symbol is found	FASTA sequence contains an unrecognized character.	

Access terms and conditions

For details on the terms involved with the IDT rhAmpSeq product portfolio, see our warranty and licenses page.

For Research Use Only. Not for use in diagnostic procedures. Unless otherwise agreed to in writing, IDT does not intend these products to be used in clinical applications and does not warrant their fitness or suitability for any clinical diagnostic use. Purchaser is solely responsible for all decisions regarding the use of these products and any associated regulatory or legal obligations.

© 2026 Integrated DNA Technologies, Inc. All rights reserved. Trademarks contained herein are the property of Integrated DNA Technologies, Inc. or their respective owners. For specific trademark and licensing information, see http://www.idtdna.com/trademarks.