



# Using the rhAmpSeq™ CRISPR Analysis Tool

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# Access the rhAmpSeq CRISPR Analysis Tool

From the IDT website, go to Tools, CRISPR Genome Editing, then select the rhAmpSeq CRISPR Analysis Tool.

The screenshot shows the IDT website header with the logo, a search bar, and links for GET HELP, SIGN IN, and a shopping cart. Below the header is a navigation bar with categories: PRODUCTS & SERVICES, APPLICATIONS & TECHNOLOGIES, SUPPORT & EDUCATION, TOOLS (highlighted with an orange circle), and COMPANY. The TOOLS dropdown menu is open, displaying various tool categories. Under the CRISPR GENOME EDITING category, the rhAmpSeq CRISPR Analysis Tool is highlighted with an orange circle. Other categories include OLIGO DESIGN & HANDLING, qPCR ASSAY DESIGN, GENE REGULATION AND RNAi, GENOTYPING, GENES & GENE FRAGMENTS, and NGS TOOLS. A 'Browse all tools' link is at the bottom left of the menu.

## Create an account

An IDT account provides access to the Analysis Lab page and the ability to use the rhAmpSeq CRISPR Analysis Tool.

The screenshot shows the IDT website with the rhAmpSeq CRISPR Analysis Tool page. The header is the same as the previous screenshot. The main content area features a large heading 'rhAmpSeq™ CRISPR Analysis Tool' with a wrench icon. Below the heading is a description: 'Flexible, cloud-based interrogation of CRISPR-mediated, double-strand break repair'. A list of bullet points describes the tool's capabilities: Analyze on- and off-target CRISPR edit sites without having bioinformatics expertise; Characterize Cas 9 or Cas12a (Cpf1) edits (contact us for other nucleases); Batch analyze singleplex or multiplex samples (≤500 targets) simultaneously; Interpret results with supported, validated, versioned workflows; Streamline analysis with same-day data delivery; Access with purchase of rhAmpSeq CRISPR Library Kit. A note at the bottom states: '\* This service is not currently available in China or Russia'. To the right of the tool description is a 'PLEASE SIGN IN' section with input fields for 'UserName' and 'Password', a checkbox for 'Keep me signed in. Details', a 'Forgot Password' link, and two buttons: 'SIGN IN' (orange) and 'REGISTER' (blue).



**Note:** If you do not already have an IDT account, click Register to create your own account.

## Account Information

### Login Information

Login Name:  \*

Password:  \*

Confirm Password:  \*

This is the information you will use to log in when you order, view past orders, or change your account information.

### Personal Information

First Name:  \*

Last Name:  \*

Email:  \*

Confirm Email:  \*

PI First Name:  \*

PI Last Name:  \*

Institution Type:  \*

Company/Institution:  \*

I'd like to receive scientific updates from IDT: ☐


I'd like to speak to IDT Sales: ☐


#### Important:

Once established, your first and last name cannot be changed without emailing [custcare@idtdna.com](mailto:custcare@idtdna.com); for organization or institution changes, we ask that you please create a new account under the new parent organization. Please contact IDT Customer Care if you have any questions or concerns.

IDT uses your contact information in order to provide you with information about our services. IDT does not share your personal information with any non-affiliated third parties, other than our distributors, nor do we process your personal information for any other purpose. To learn more about how IDT treats your information, please review our [privacy statement](#).

Once established, you will use this account login to sign in and use the tool.






[GET HELP](#)
[SIGN IN](#)

[0 ITEMS \\$0.00 USD](#)

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### Please Sign In





☐ Keep me signed in. [Details](#)
[Forgot Password](#)

[SIGN IN](#)
[REGISTER](#)

Please sign in to use IDT's custom online ordering tools. If you don't yet have an IDT account, join the IDT community! Create your free account today and enjoy unlimited access to our innovative web tools, streamlined ordering, and expert educational content.

After you log in, the Analysis Lab landing page opens. If you are not already part of an analysis lab, click the Create a Lab button and give a unique name to your lab.



## Analysis Lab Overview

You don't belong to any active lab. Please join or create one.

[CREATE A LAB](#)[REDEEM CREDIT](#)[SEND INVITE](#)[ANALYSIS TOOL](#)

### Create New Lab

Lab Name

[CREATE](#)

After creating a lab, you can invite collaborators, colleagues, and students to join your analysis lab by clicking Send Invite. Enter a list of those you want to collaborate with, and a custom message to accompany your invitation.

## Analysis Lab Overview

CRISPAItRationsBFXSupport

Analysis Credit Balance: 1000

[Manage lab](#)[CREATE A LAB](#)[REDEEM CREDIT](#)[SEND INVITE](#)[ANALYSIS TOOL](#)[Members](#)[Pending invites](#)

Member Name

Gavin Kurgan

### Send Lab Invite(s)

Send to

Invite message

[SEND](#)

**Tip:** You can control user privileges by setting permission levels for each invited member.

## Manage your account

To use the rhAmpSeq CRISPR Analysis Tool, you will need Analysis Credits. You received a code for these credits when the order for your purchased product was invoiced. To redeem your Analysis Credits, click the gray **Redeem Credit** button, then enter the code. View your current balance and transaction history in the top left corner.



**Tip:** Refresh the page to see updated information on your account balance.

## Analysis Lab Overview

CRISPAItRationsBFXSupport

Analysis Credit Balance: 1000

[VIEW RECENT TRANSACTIONS](#)[Manage lab](#)[CREATE A LAB](#)[REDEEM CREDIT](#)[SEND INVITE](#)[ANALYSIS TOOL](#)[Members](#)[Pending invites](#)

Member Name

Gavin Kurgan

Ellen Black


### Redeem Credit

Credit code

[REDEEM](#)

You are about to leave IDT's website to access IDT's rhAmpSeq CRISPR Analysis Tool hosted on BlueBee, Inc.'s website. BlueBee is a third party service provider to IDT. The Terms and Conditions, IDT Privacy Policy, and the Data Protection Addendum governing your use of IDT's rhAmpSeq CRISPR Analysis Tool can be found [here](#) and [here](#) and [here](#).

**CANCEL** **CONFIRM**

 **Tip:** Since you have learned how to create and manage your account, you are ready to explore the other [tutorial videos](#) that further describe how to use the IDT rhAmpSeq CRISPR Analysis Tool.

## Upload data

- read name
- sequence
- associated quality score

[illegible]

There are a few different ways to upload your data to the rhAmpSeq CRISPR Analysis Tool.

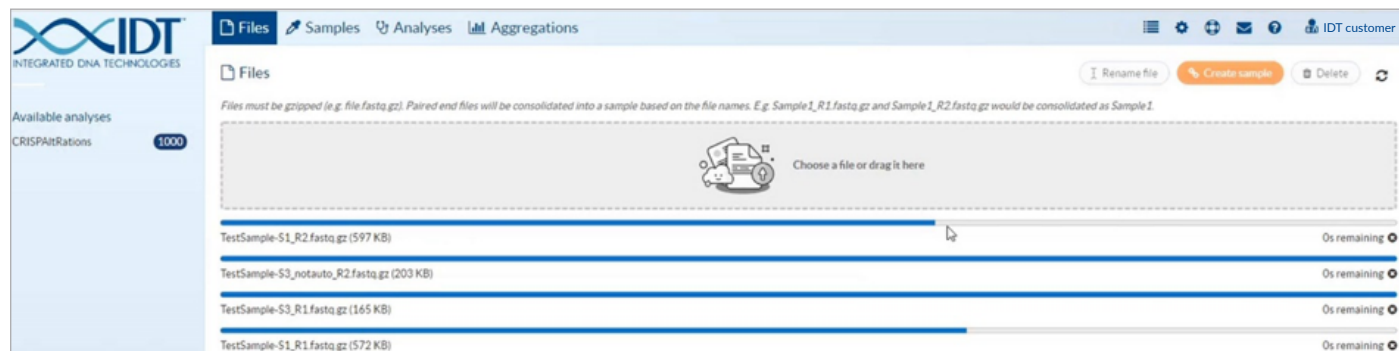
You can:

- drag and drop files into the application
- upload files from a local drive
- upload files from the cloud using the Connector

For smaller file sizes, using drag-and-drop is the fastest and easiest method for uploading sequence data.

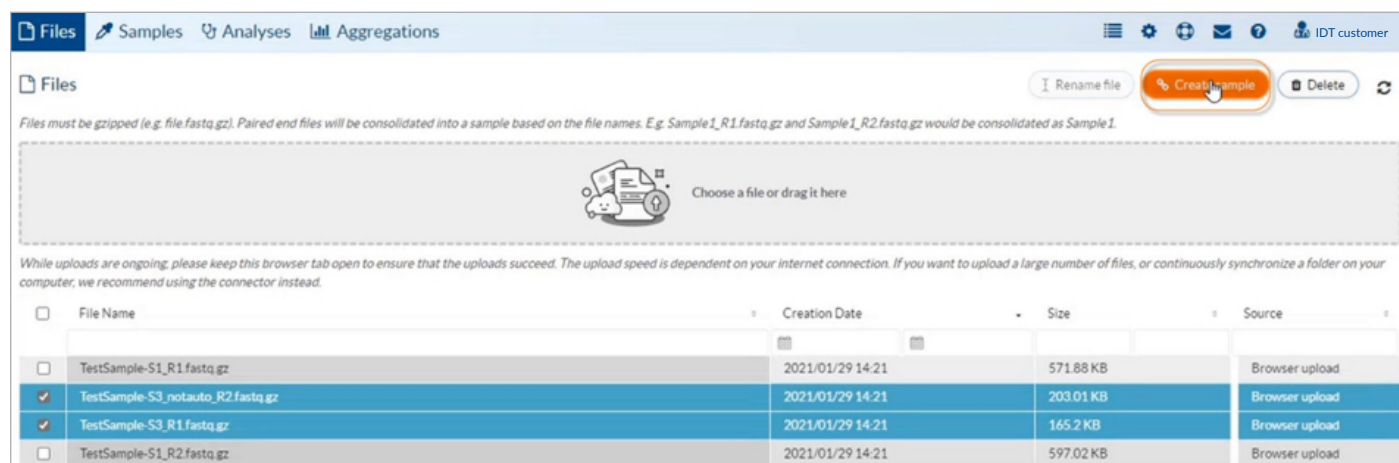
Simply navigate to the **Files** tab, then drag the samples you want analyzed into the open gray box in the interface as shown below.

Files are uploaded in parallel, and upon successful upload, R1/R2 files are automatically paired to create a "Sample."



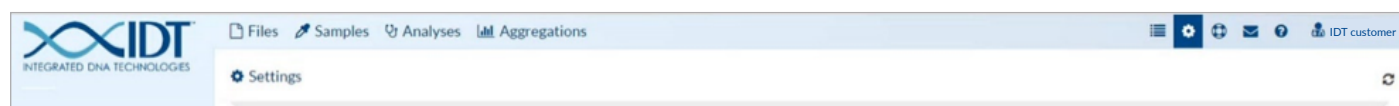
**Note:** For drag-and-drop uploads you must stay on the upload page until the transfer is completed. Wait for the progress bars to indicate when the uploads are complete before moving to the next step.

**Note:** Alternatively, you can manually pair read data into a sample by selecting the uploaded files, then clicking the orange **Create sample** button.



## Upload large files via Connector setup

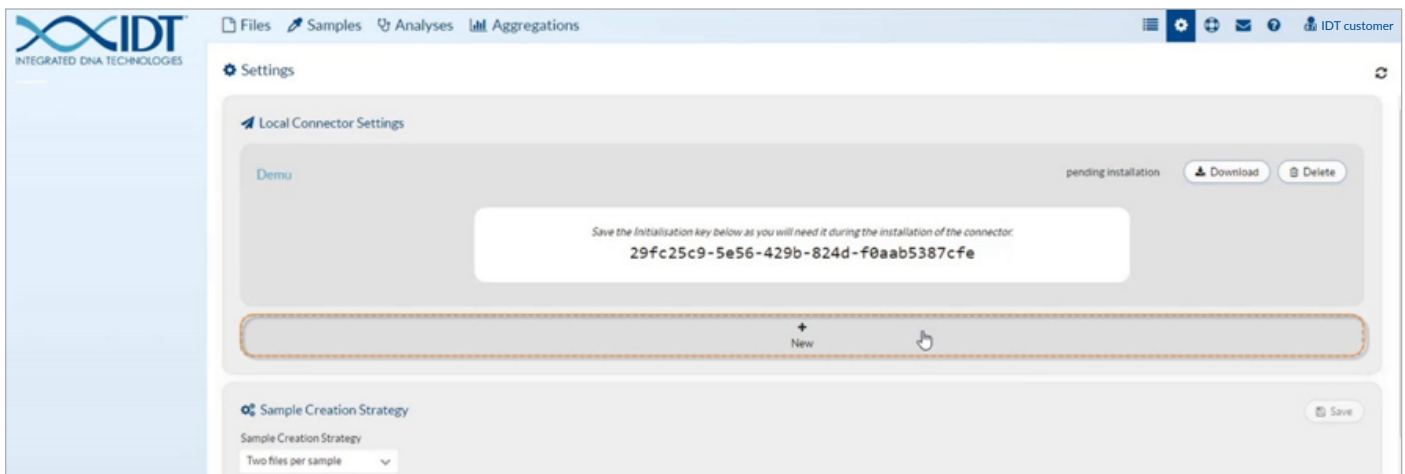
For larger files, or high-throughput applications, the drag-and-drop method may not be fast enough. Consider using the Connector setup upload feature. Navigate to the **Settings** page, indicated by a gear icon.



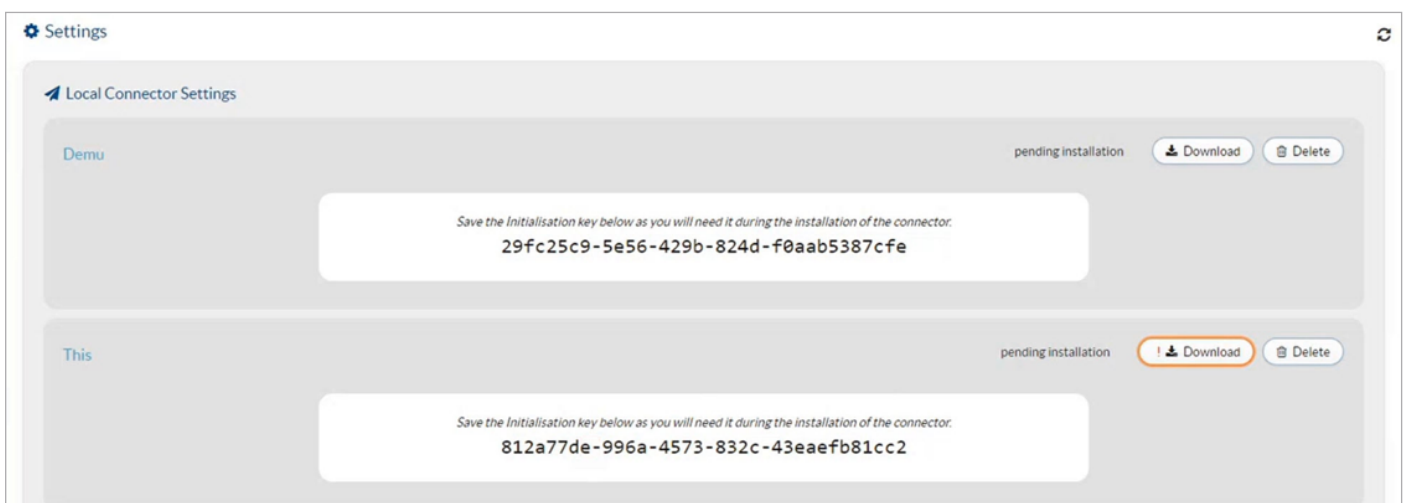
Here, you can set up the uploading of data from your local drive, or cloud storage systems. This guide will give you a high level overview of how to set up the Connector; for a more in-depth review, see the [rhAmpSeq CRISPR Analysis Tool Data Transfer Guide](#).

To start uploads from your local drive to the analysis platform, click the **New** button under Local Connector Settings, then specify the following:

- name of the connector
- computer's operating system
- upload/download location of your data



Save your inputs, then download and install the connector software.



Follow the on-screen installation prompts.

To start uploads from a cloud streaming scenario, click the **New** button under the appropriate cloud service provider that you use. The setup is the same as above (uploads from a local drive), except that some specifics for each cloud provider may differ. Reference the [rhAmpSeq CRISPR Analysis Tool Data Transfer Guide](#) for detailed instructions based on your cloud provider.

# Data with multiple amplicons

If you are analyzing data with multiple amplicons in each sample, you will need to upload two 6-column BED-formatted files specifying amplicon and guide coordinates of the genome of interest. These BED files contain the following for all targets being analyzed:

- chromosome
- start location
- stop location
- name
- strand

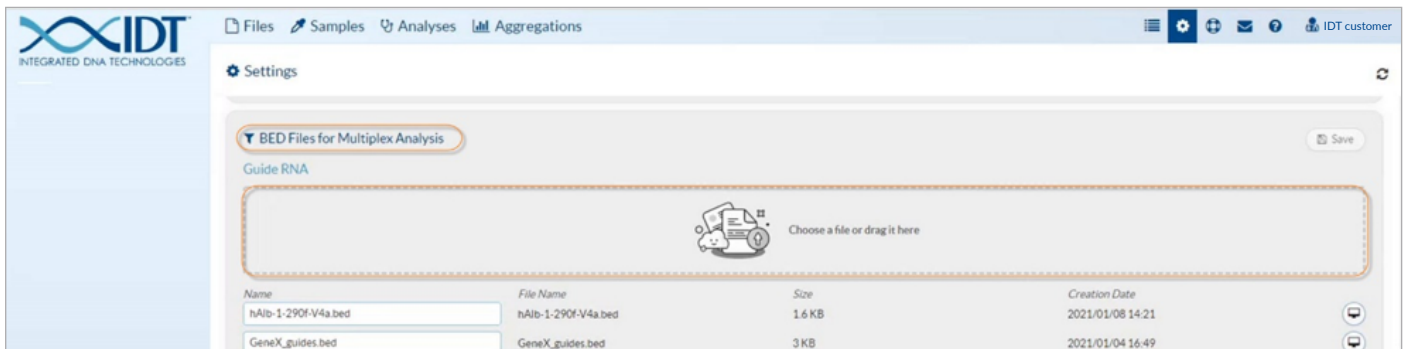
**!** **Important!** Guide coordinates should not include the protospacer adjacent motif (PAM) but should include the correct strand information (+ or -) so that the guide is 5' to 3'.

**☰** **Note:** If the [rhAmpSeq Design Tool](#) was used to generate the panel, then the amplicon BED file can be found in the Design Tool dashboard, as shown below. The file "Assay\_panel.bed" will work for this.

View of GeneX_guides.bed					
File Name		GeneX_guides.bed		Size	3 KB
				Creation Date	2021/01/04 16:49
1	chr11	36573321	36573341	GeneX	0 -
2	chr1	3592654	3592674	OT1	0 -
3	chr1	16415506	16415526	OT2	0 +
4	chr1	33698343	33698363	OT3	0 +
5	chr1	34144141	34144161	OT4	0 +
6	chr1	49123705	49123725	OT5	0 -
7	chr1	52927734	52927754	OT6	0 +
8	chr1	115105040	115105060	OT7	0 -
9	chr1	154428110	154428130	OT8	0 +
10	chr1	166894026	166894046	OT9	0 -
11	chr1	183144152	183144172	OT10	0 -
12	chr1	200374008	200374028	OT11	0 -
13	chr1	206221475	206221495	OT12	0 +
14	chr10	6437025	6437045	OT13	0 -
15	chr10	106920200	106920220	OT14	0 -
16	chr10	121109396	121109416	OT15	0 -
17	chr10	125696309	125696329	OT16	0 +
18	chr11	2525645	2525665	OT17	0 +
19	chr11	12125984	12126004	OT18	0 -
20	chr11	20102285	20102305	OT19	0 +
21	chr11	57348913	57348933	OT20	0 -
22	chr11	64787970	64787990	OT21	0 -
23	chr11	103863224	103863244	OT22	0 -
24	chr12	26976538	26976558	OT23	0 +
25	chr12	49647269	49647289	OT24	0 -
26	chr12	56088641	56088661	OT25	0 +
27	chr12	57198911	57198931	OT26	0 +
28	chr12	57289518	57289538	OT27	0 +
29	chr12	74842086	74842106	OT28	0 +
30	chr12	90948235	90948255	OT29	0 -
31	chr12	106046735	106046755	OT30	0 +
32	chr12	109286629	109286649	OT31	0 -
33	chr12	124489776	124489796	OT32	0 +
34	chr13	22458937	22458957	OT33	0 +

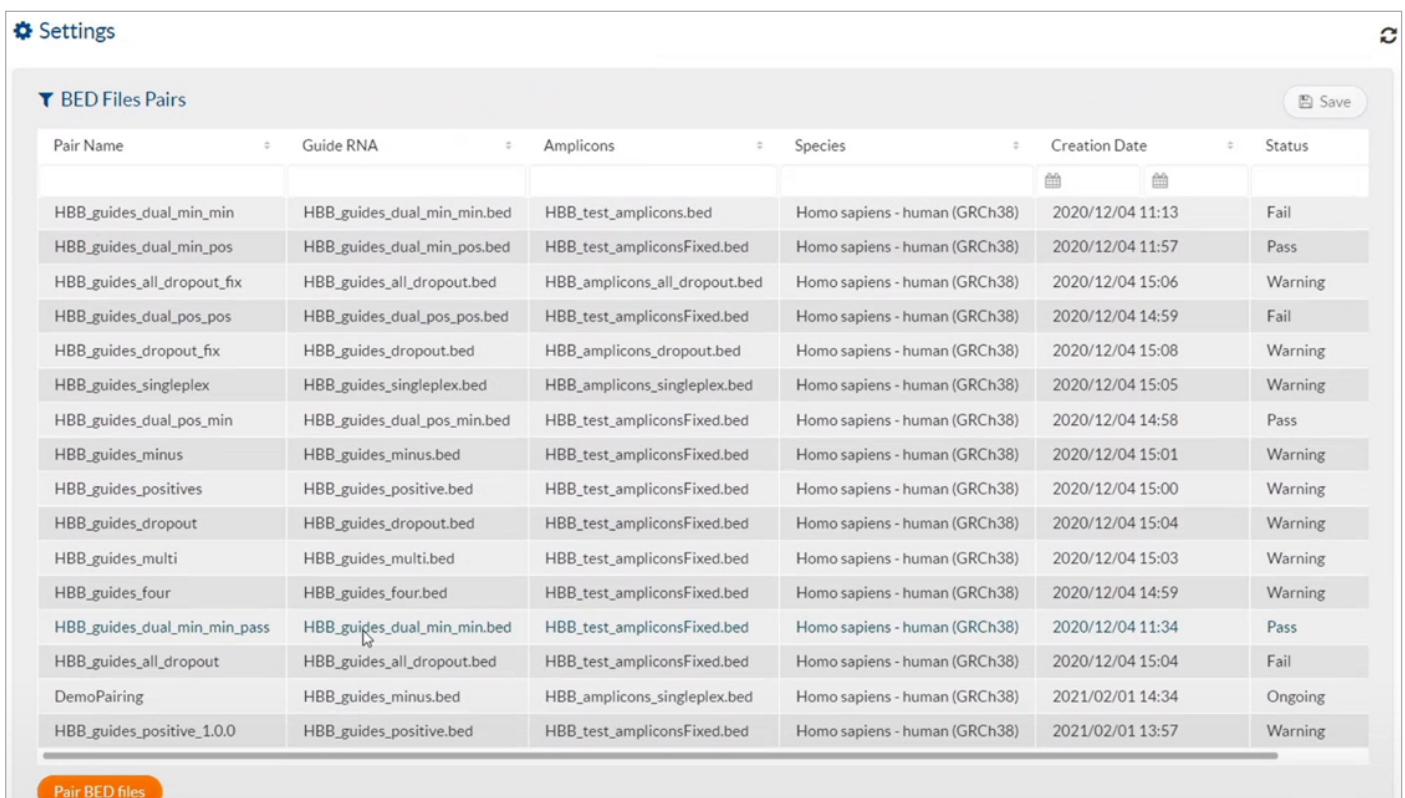


To upload these files, use the gear icon to open the **Settings** page, then scroll down to find the section called “BED Files for Multiplex Analysis.” Upload your guide RNA and amplicon BED files into the specified sections by using the drag-and-drop upload method.



## Quality control checking BED inputs

In order to use your BED files for multiplex analysis, the uploaded BED files need to be checked for errors. Navigate to the **Settings** page, then look for the “BED Files Pairs” section. This is where you QC-check your inputs to make sure they can successfully be used for analysis. To begin, select the orange **Pair BED files** button, as shown below.



You will enter:

- a unique name to reference the files being paired
- the pipeline version intended for analysis
- guide and amplicon BED files
- species these coordinates come from

These entries will initiate the QC check on the files, which will take approximately 5–10 minutes to complete. A status message of PASS, WARNING, or FAIL will appear when the pairing is complete.

```
02/01/2021 08:20:49 PM - INFO - create:set_up_logging - Logging to bedQC.log
02/01/2021 08:20:49 PM - WARNING - columnBiasCheck:columnBiasCheck - There is only one value of {'+'} in column 5; make sure this
02/01/2021 08:20:50 PM - INFO - create:set_up_logging - Logging to bedQC.log
```

## Handling QC statuses

If a **PASS** status is shown, you are ready to start your analysis.

If a **WARNING** status is shown, double-click the pairing to understand why the paired BED files were flagged. These files will still be available for analysis; however, they should be reviewed to make sure there are no persistent problems with your experimental inputs.

If a **FAIL** status is shown, there is something wrong with the BED inputs that needs correction. Check the log and make the appropriate corrections in the files to reverse the failure.

**Important!** Once paired, BED files cannot be deleted from the analysis tool. This is to ensure they are available for previous analyses that used them. Try to provide unique and memorable names for these BED file pairs.

## Analyzing singleplex experiments

If you want to screen on-target guides and conditions, you will need to know how to analyze your singleplex experiment.

**Important!** Make sure that data has been completely uploaded and put into samples before proceeding.

First, select your samples. Navigate to the **Samples** page where you will be able to see all the samples that have FASTQ files associated with them. Select the groups of samples with a single amplicon that you want to analyze. Begin the analysis by clicking **Analyze Singleplex**.

To start the run, you will need to associate information and parameters with the run and individual samples. This is where you will also specify the version of the CRISPAItRations software to use for the analysis.

The screenshot shows the 'Samples' page in the CRISPAItRations software. The 'Run Singleplex Analysis' dialog box is open, displaying the following information:

- Selected samples (3):** Singleplex\_GeneX\_Control, Singleplex\_GeneX\_EditedHDR and Singleplex\_GeneX\_EditedOnly
- Analysis mode:** CRISPAItRations 1.0.0 (selected from a dropdown menu)
- Analysis Tags:** Type to add a tag (input field with a dropdown arrow)

Buttons for 'Analyze Singleplex', 'Analyze Multiplex', 'Edit', 'Unlink Files', 'Delete', and 'Run Singleplex Analysis' (with 'Analyze' and 'Cancel' sub-buttons) are visible.

Before starting the run, provide the information highlighted by red boxes, namely the nucleotide sequences for the guide and the amplicon, 5' to 3', excluding the PAM.

Sample	Guide Sequence	Amplicon Sequence	Nuclease	Window Size	Donor
Singleplex_GeneX_Control	<input type="text"/>	<input type="text"/>	Cas9	default	<input type="text"/>
Singleplex_GeneX_EditedHDR	<input type="text"/>	<input type="text"/>	Cas9	default	<input type="text"/>
Singleplex_GeneX_EditedOnly	<input type="text"/>	<input type="text"/>	Cas9	default	<input type="text"/>

The additional parameters that can be configured on a run include:

- the applicable CRISPR nuclease
- window size for interrogating variants
- nucleotide sequences for HDR donors
- well position (for advanced visualizations)
- sample name
- treated vs. untreated sample

**Note:** You can quickly populate the same entry down an entire column at once by entering the information in the first row, then clicking the down arrow icon to the right of the field.

**Note:** For batch runs that have a large number of samples, you will need to upload a samplesheet to provide run information. To get a template of this samplesheet, click the **Export to CSV** button.

AutoSave Off

GeneX\_Samplesheet\_commaExample - Excel

Sample	Guide Sequence	Amplicon	Nuclease	Window S	Donor	Well	Group ID	Treated
Singleplex_GeneX_Control	GGCATCGTGACTACACGG	GCTCAGA	Cas9	default		A1	Control	FALSE
Singleplex_GeneX_EditedHDR	GQCATCGTGACTACACGG	GCTCAGA	Cas9	default	ATGGTCTA3		EditedHDI	TRUE
Singleplex_GeneX_EditedOnly	GGCATCGTGACTACACGG	GCTCAGA	Cas9	default		A2	Edited	TRUE

After specifying your sample information in Excel, save and upload this file directly into the interface using the **Import from CSV** button.

Before starting your run, designate an "Aggregation" name, which creates a single location to interrogate all samples in the run. You can add additional metadata and unique descriptions to help label samples and experimental details for tracking results before starting the run.

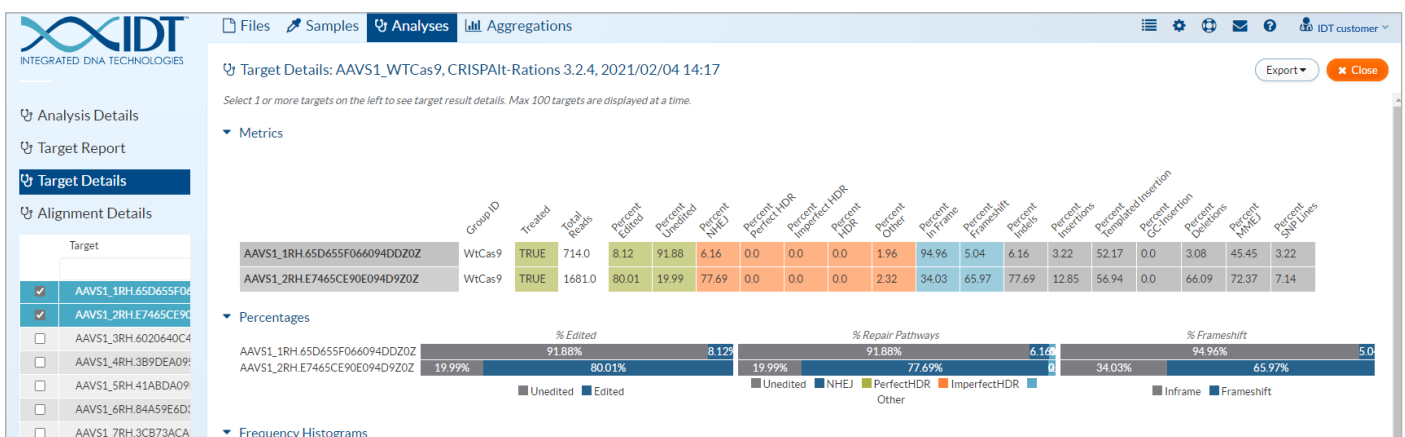
**Caution:** When a red box highlights any of the sample information fields, you will not be able to start the run. Check the hover text on any parameter with a red box to find acceptable inputs and make corrections accordingly. When all inputs are satisfied and no red boxes appear, then you are ready to begin the run.



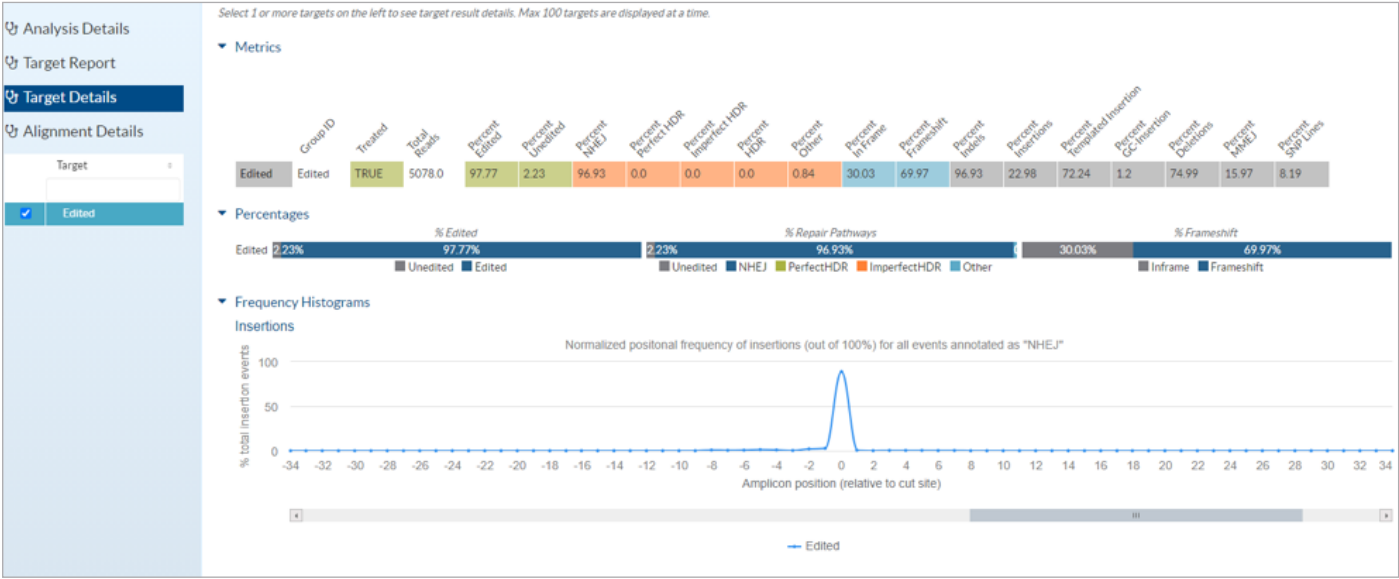
To check the status or results of a run, navigate to the **Analyses** page. As soon as cloud resources begin processing, a progress bar should appear. Analyses can take between 20 mins–2 hrs to complete.

## Interpreting singleplex experiments

View your results by double-clicking the sample on the **Analyses** page. This opens the landing page where you will interpret the results of your experiment. View the graphics for all targets amplified in the “Target Details” section. You can also easily export the graphics in the interface for selected targets using the **Export** button in the top right corner of the page.

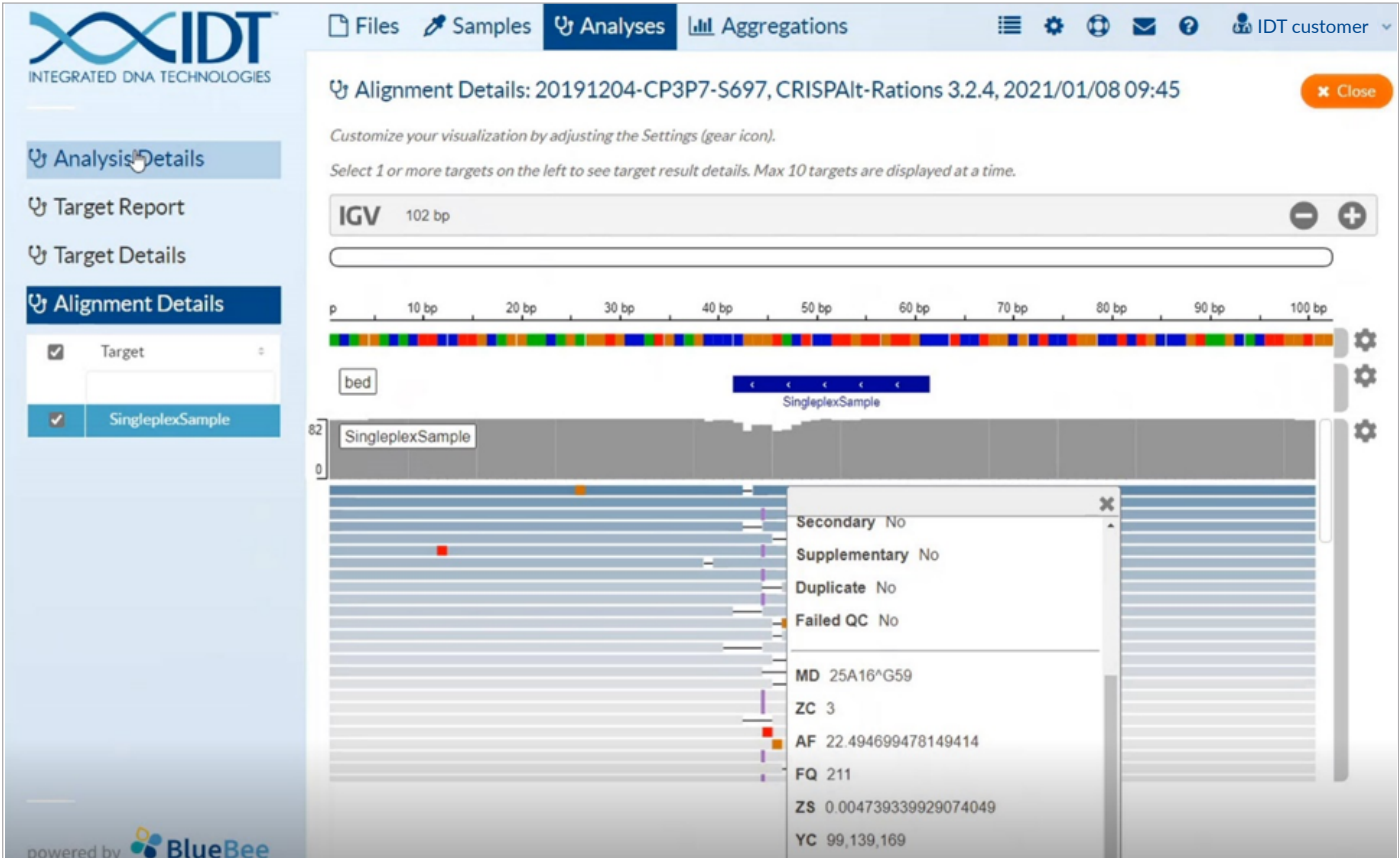


Also, on this page you will be able to find the frequency of the editing, the specific repair pathways used, and any frameshifting events that may have occurred. Dive deeper to explore the exact position of insertion and deletion events in the sequenced sample, even the size of these events. We include a frequency histogram showing the normalized positional frequency of insertions, as shown below.



## Viewing allele details

Go to the **Alignment Details** page to view the distribution of alleles in the sequenced population using the Integrative Genomics Viewer (IGV). Click on the event of interest to see both the frequency of the event and the read count.



For those with advanced graphical considerations, the raw data we use to render the graphics can be downloaded from the Analysis Details page.

Analysis Details

Target Report

Target Details

Alignment Details

Guide Sequence

GGCATCGTGACTACCACGG

Nuclease

Cas9

Max Overlap

152

Min Read Size

60

Window Size

default

Group ID

Edited

Treated

TRUE

Species

notSpecified

Total on-target reads

5,078

% Mapped

99.84

Reads (total)

5170.0

% QC passing

99.45841

% Merged

98.91093

% Primer-dimer

0.0

% On-target

99.84

Donor

Well

A2

Results

File Name	Size	Format
OUTPUT.tar.gz	821.27 KB	UNKNOWN
SAMPLE_allSnpsTables.csv	11.74 KB	CSV
SAMPLE_AllTargetEditing.png	11.69 KB	PNG
SAMPLE_collapsedIndelInfo.csv	305.19 KB	CSV
SAMPLE_deleteLocationHist.csv	8.14 KB	CSV
SAMPLE_indel.csv	2.96 KB	CSV
SAMPLE_insertLocationHist.csv	5.62 KB	CSV
SAMPLE_summary.csv	704 B	CSV
SUMMARIES.tar.gz	17.94 KB	UNKNOWN

Results files of the targets are consultable in the Target Details menu.

On this page, you can see general sample information, as well as an assortment of other files for either viewing or downloading.

Files

Samples

Analyses

Aggregations

Analysis Details: 20191204-CP3P7-S697, CRISPAIt-Rations 3.2.4, 2021/01/08 09:45

total on-target reads 12775

Analysis Details

Target Report

Target Details

Alignment Detail

View of SAMPLE\_collapsedIndelInfo.csv

File Name

SAMPLE\_collapsedIndelInfo.csv

Size

23.99 KB

Creation Date

2021/01/08 09:55

freqCount	ReferenceAlignment
211	ACAGGACACTTCCTTGCAGGAACAGAGGTGCCATGCAGCCCCGGGTACTCCTTGTGTTGCC
109	ACAGGACACTTCCTTGCAGGAACAGAGGTGCCATGCAGCCCCGGGTACTCCTTGTGTTGCC
69	ACAGGACACTTCCTTGCAGGAACAGAGGTGCCATGCAGCCCCGGGTACTCCTTGTGTTGCC
69	ACAGGACACTTCCTTGCAGGAACAGAGGTGCCATGCAGCCCCGG--GTACTCCTTGTGTTGCCCTCTGGCGCTCTGGCCCTCTGCCCGTAAGCACTTGGTGG
44	ACAGGACACTTCCTTGCAGGAACAGAGGTGCCATGCAGCCCCGGGTACTCCTTGTGTTGCC
43	ACAGGACACTTCCTTGCAGGAACAGAGGTGCCATGCAGCCCCGG--

Close

Format

UNKNOWN

CSV

PNG

CSV

CSV

CSV

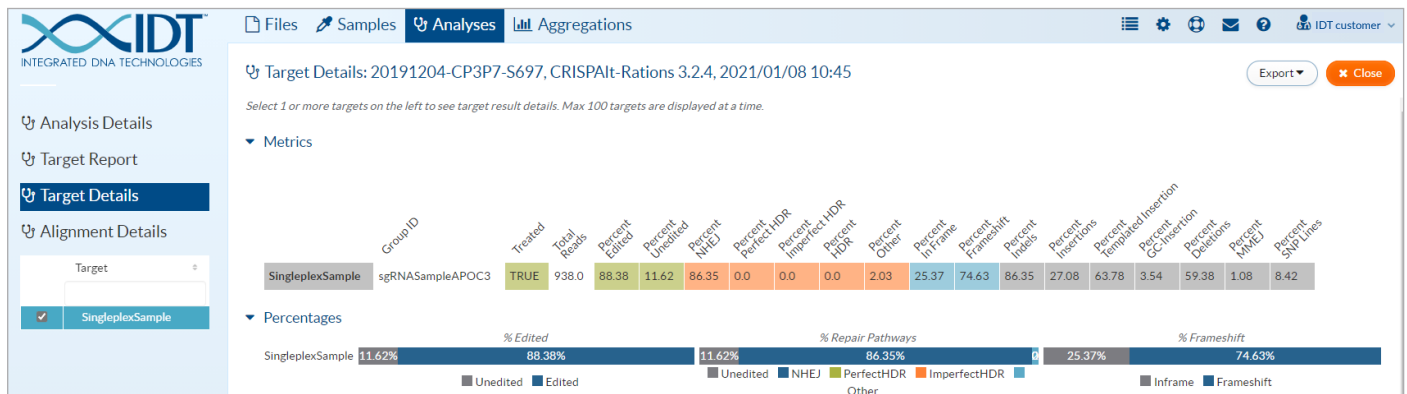
CSV

# Viewing the target report

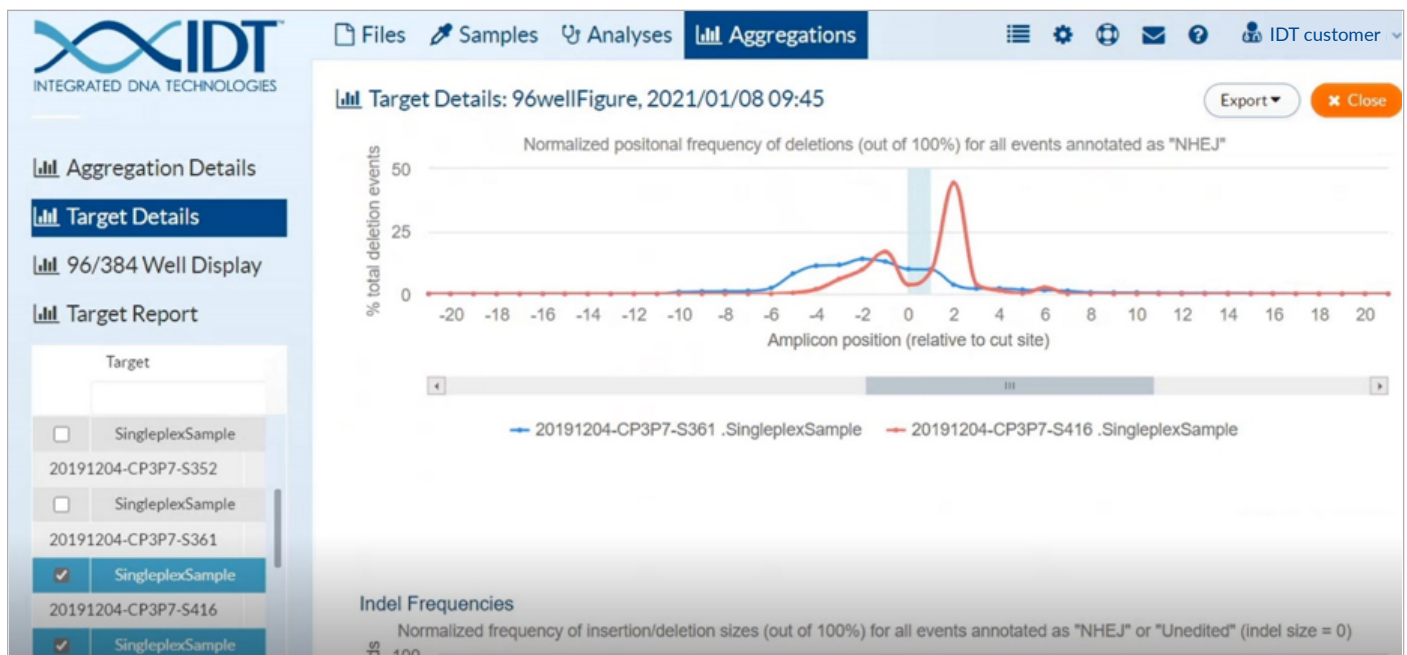
To interpret and compare results of different samples within an experiment, go to the **Aggregations** tab.

On the **Target Report** landing page, view all the samples that were aggregated for summarization. Any report that you view can easily be exported to Microsoft Excel. From the **Target Report** page, sort or filter the experimental results as needed, and create additional graphics in the software of your choice.

To compare a sample with another, click **Target Details**. On this page you will find the same graphics that were available for individual samples; however, here you can also overlay results from other relevant samples within the whole experiment.



In the graphic below, the run contained two samples with the same guide, treated under different conditions. Notice that although the editing efficiency is different, the overall indel profile of these two samples is similar, as expected.

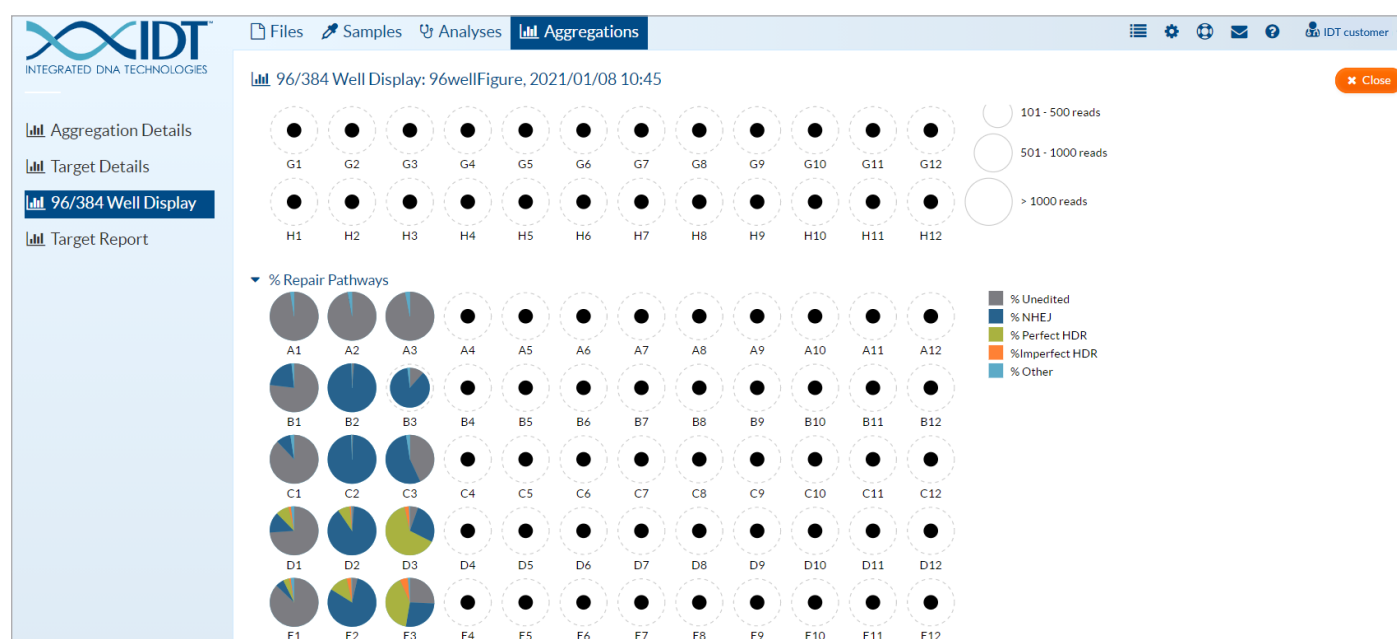


# Screening guides

When screening a large number of guides under varying conditions and treatments, you can generate graphics in 96- or 384-well plate formats to find conditions that are optimal for your experimental goals. The graphics in 96- or 384-well plate formats are only available for singleplex analyses. To create these graphics, simply specify the “Well” information for each singleplex sample prior to sample analysis.

Once your run is complete, go to the **Aggregations** tab where you can find graphics displaying the following:

- frequency of editing
- repair pathway utilization
- frameshift frequency



## Analyzing multiplex experiments

The most common application for multiplex CRISPR NGS is to quantify on- and off-target editing effects of a guide. In our example, we show how to analyze samples that have multiple amplicons per sample, known as multiplexed samples.

**Important:** Make sure the following have been completed before proceeding.

- Data have been uploaded and created into samples
- Guide and amplicon BED files have been uploaded and paired

**Tip:** For a deep dive into pairing your guide and amplicon BED files, watch the section of the rhAmpSeq CRISPR Analysis Tool video [How to quality control check multiplex file inputs](#).



To begin, select the samples you want to analyze, then click **Analyze Multiplex**.

The screenshot shows the 'Samples' page in the IDT interface. At the top, there are tabs for 'Files', 'Samples', 'Analyses', and 'Aggregations'. Below the tabs, there are buttons for 'Analyze Singleplex', 'Analyze Multiplex' (highlighted with a mouse cursor), 'Edit', 'Unlink Files', and 'Delete'. A message says 'Select all samples you wish to analyze together.' Below this is a table with columns: Sample Name, Creation Date, Status, Size, and Experiment. Three samples are listed, all with a status of 'New' and a size of 2.74 MB, 3.13 MB, and 2.18 MB respectively. The 'Experiment' column shows 'tutorial'.

Sample Name	Creation Date	Status	Size	Experiment
AAVS1_Ctrl	2021/02/02 15:36	New	2.74 MB	tutorial
AAVS1_WTCas9	2021/02/02 15:35	New	3.13 MB	tutorial
AAVS1_HiFiCas9	2021/02/02 15:35	New	2.18 MB	tutorial

Once your data has successfully been checked for quality control, select your named guide/amplicon pairs and other relevant run information, including:

- the appropriate CRISPR nuclease
- window size for interrogating variants
- nucleotide sequences for HDR donors
- sample name information
- treated vs. untreated

Navigate to the **Analyses** page to check the status or results of your analysis. As soon as cloud resources begin processing, you will see the progress bar. Analyses can take anywhere from 20 mins–2 hrs to complete. Your turnaround time will depend on your read depth plus the number of amplicon targets being quantified per sample.

The screenshot shows the 'Analyses' page in the IDT interface. At the top, there are tabs for 'Files', 'Samples', 'Analyses', and 'Aggregations'. Below the tabs, there are buttons for 'Edit' and 'Aggregate'. A message says 'Click the sample name to view analysis results of a single sample.' Below this is a table with columns: Sample, Progress, Status, Reads (total), % QC passing, and % Merged. The table lists 16 samples, all with a status of 'success' and a progress bar of 100%. The 'Reads (total)' column shows values ranging from 4,561 to 39,934. The '% QC passing' column shows values ranging from 99.50957 to 99.82619. The '% Merged' column shows values ranging from 98.79622 to 99.82619.

Sample	Progress	Status	Reads (total)	% QC passing	% Merged
20191204-CP3P7-S978	100%	success	4,561	99.76108	99.629875
20191204-CP3P7-S923	100%	success	33,598	99.65163	99.54672
20191204-CP3P7-S914	100%	success	10,308	99.50957	99.623116
20191204-CP3P7-S71	100%	success	6,047	99.523575	99.83493
20191204-CP3P7-S80	100%	success	21,694	99.619705	99.79303
20191204-CP3P7-S1259	100%	success	5,841	99.81324	99.64279
20191204-CP3P7-S135	100%	success	3,389	99.735374	99.97052
20191204-CP3P7-S416	100%	success	3,398	99.82619	99.44863
20191204-CP3P7-S352	100%	success	8,849	99.54041	99.71847
20191204-CP3P7-S361	100%	success	32,353	99.60705	99.71336
20191204-CP3P7-S642	100%	success	38,877	99.583984	99.64375
20191204-CP3P7-S1204	100%	success	39,934	99.646835	99.67803
20191204-CP3P7-S697	100%	success	2,275	99.82832	98.79622
20191204-CP3P7-S1195	100%	success	4,938	99.53704	99.858444
20191204-CP3P7-S633	100%	success	7,462	99.469284	99.573166

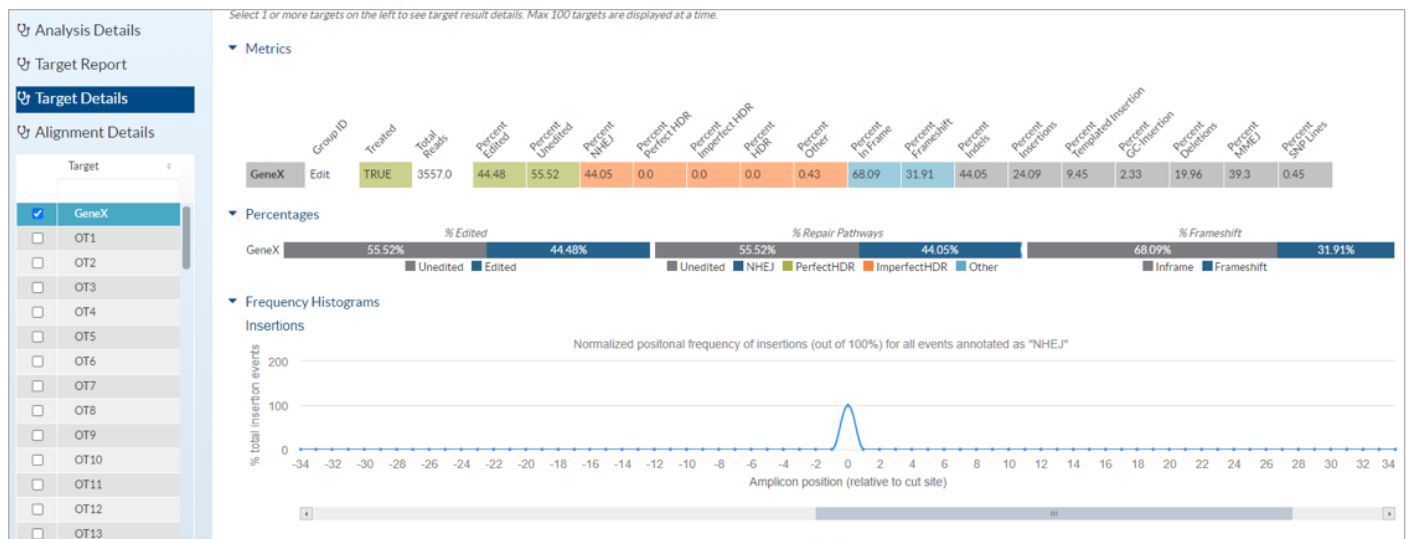
# Interpreting multiplex experiments

When you have completed your analysis, the **Target Details** landing page displays. This page contains graphics for all amplified targets. Each target can be toggled on or off by checking or unchecking the checkboxes to the left of the targets in the table. Also, the active display on the page can easily be exported by using the **Export** button in the top right corner.

From the **Target Details** page, you can find a range of data, including:

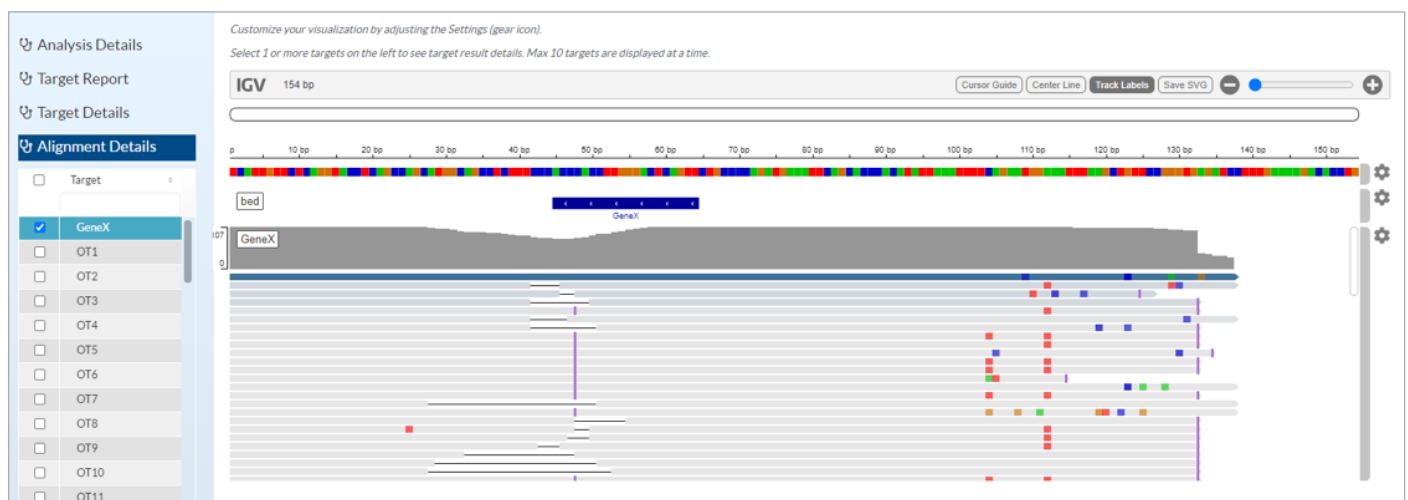
- frequency of editing
- repair pathways
- frameshifting events

Additionally, you can explore the position of any insertion and deletion event that occurred in the sequenced sample and the size of these events. We have included a frequency histogram that shows the normalized positional frequency of insertions below.



## Viewing allele details

Go to the **Alignment Details** page to view the distribution of alleles in the sequenced population using IGV. Click on the event of interest to see both the frequency of the event and the read count.



For those with advanced graphical considerations, the raw data we use to render these graphics can be downloaded from the **Analysis Details** page. On this page, you can see general sample information, as well as an assortment of other files for either viewing or downloading.

**Analysis Details**  
Target Report  
Target Details  
Alignment Details

Guide RNA: GeneZZ\_guides.bed  
Amplicons: GeneZZ\_amplicons.bed  
Donor:   
Well: NotSpecified  
Nuclease: Cas9  
Max Overlap: 152  
Min Read Size: 60  
Window Size: default  
Group ID:   
Treated: TRUE  
Species: Homo sapiens - human (GRCh38)  
Total on-target reads: 363,891  
Reads (total): 364,000.0  
% Merged: 99.970604  
% On-target: 100.0  
% Mapped: 100  
% QC passing: 99.99945  
% Primer-dimer: 0.0

**Results**  

File Name	Size	Format		
OUTPUT.tar.gz	108.8 MB	UNKNOWN		
SAMPLE_allSnpsTables.csv	764.44 KB	CSV		
SAMPLE_AllTargetEditing.png	21.41 KB	PNG		
SAMPLE_collapsedIndelInfo.csv	1.29 MB	CSV		
SAMPLE_deleteLocationHist.csv	344.52 KB	CSV		
SAMPLE_indel.csv	21.91 KB	CSV		
SAMPLE_insertLocationHist.csv	338.14 KB	CSV		
SAMPLE_summary.csv	14.29 KB	CSV		
SUMMARIES.tar.gz	109.36 KB	UNKNOWN		

Results files of the targets are consultable in the Target Details menu.

## Viewing the aggregated results

Go to the Aggregations tab to interpret and compare results of different samples within an experiment.

INTEGRATED DNA TECHNOLOGIES

Files Samples Analyses **Aggregations**

IDT customer

**Aggregations**

Edit Delete

Click the name of the aggregation to view its results.

<input type="checkbox"/>	Name	Creation Date	Analyses	Tags
<input type="checkbox"/>	Multiplex	2021/02/02 15:48	3	Demo
	AAVS	2021/02/02 15:45	3	multiplex
	GeneXExample	2021/01/29 11:25	3	GeneX
	AAVS_Multiplex	2021/01/29 09:56	3	Demo
	MultiplexAAVS	2021/01/29 09:55	3	Demo
	RAG1_GK	2021/01/15 10:28	2	IDT customer
	TestNonsense	2021/01/15 09:44	8	CustomerTest
	CustomerLab	2021/01/14 15:37	2	CustomerLab
	CustomerNonsense	2021/01/08 13:32	4	IDT
	96wellFigure	2021/01/08 09:45	15	96wellFigure



On the **Target Report** landing page, view all the samples that were aggregated for summarization, then export this information easily to Excel. With Excel, you can easily sort or filter experimental results as needed, then render additional graphics in the software of your choice.

The screenshot shows an Excel spreadsheet titled 'Target\_Report\_Multiplex\_2021\_02\_02\_15\_48 (2) - Excel'. The 'Data' tab is active. The spreadsheet displays a table with the following columns: Sample, Group ID, Treated, Well, Analysis mode, Guide RNA, Amplicons, and Nuclease. The data rows show multiple 'AAVS1\_Ctrl' samples, all with 'Control' Group ID, 'FALSE' Treated status, and 'NotSpecified' Well. A 'Sort' dialog box is open, showing the following settings:

- Sort by: Target
- Then by: Group ID
- Then by: Treated
- Sort On: Cell Values
- Order: A to Z

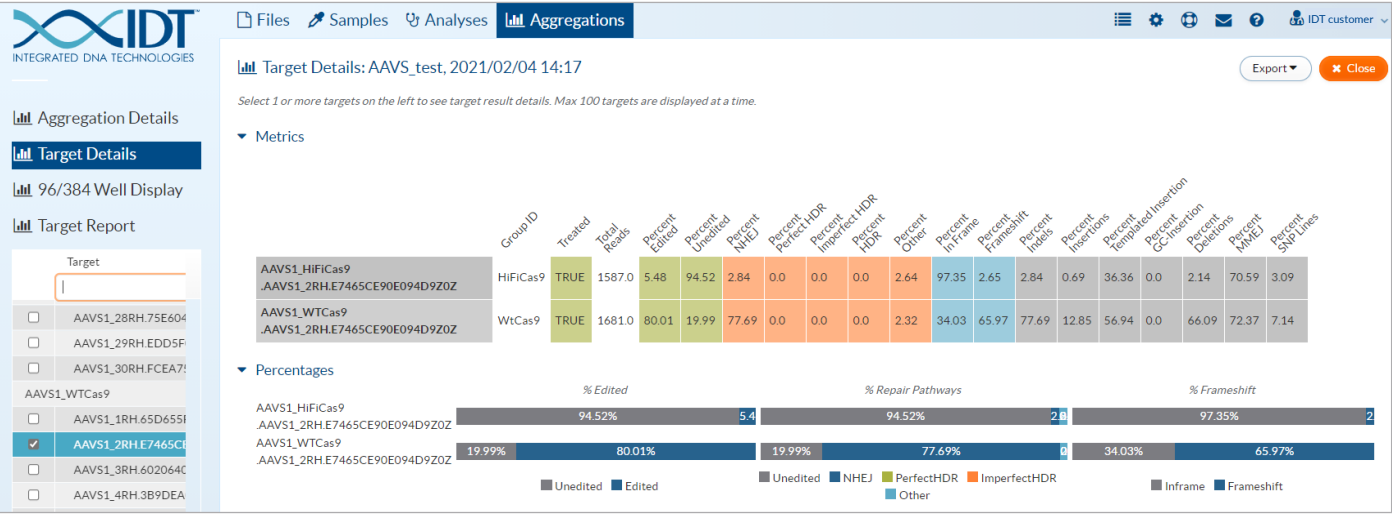
For on- and off-target editing experiments, this summary can be especially powerful for comparing treatment and control experiments. You can even use numbers in this table to implement a statistical test which can determine significant off-target effects in the experiment.

A quick sort by **Target**, **Group ID**, and **Treated** can quickly pair treatments with their corresponding controls to visualize editing differences using the conditionally formatted output.

The screenshot shows an Excel spreadsheet titled 'Target\_Report\_96wellFigure\_2021\_01\_08\_09\_45 (4) - Excel'. The 'Data' tab is active. The spreadsheet displays a table with the following columns: target, Total Reads, Percent Edited, Percent Unedited, Percent NHEJ, Percent Perfect HDR, Percent Imperfect HDR, and Percent. The data rows show various 'singleplexSample' entries with numerical values for each metric, color-coded for readability.

target	Total Reads	Percent Edited	Percent Unedited	Percent NHEJ	Percent Perfect HDR	Percent Imperfect HDR	Percent
singleplexSample	8605.00	99.36	0.64	98.80	0.00	0.00	
singleplexSample	7235.00	98.58	1.42	98.18	0.00	0.00	
singleplexSample	10023.00	98.67	1.33	89.13	7.88	1.22	
singleplexSample	938.00	88.38	11.62	86.35	0.00	0.00	
singleplexSample	4850.00	96.31	3.69	80.19	12.56	3.18	
singleplexSample	1673.00	56.96	43.04	54.45	0.00	0.00	
singleplexSample	3511.00	94.53	5.47	26.94	63.94	3.08	
singleplexSample	4655.00	74.24	25.76	26.90	40.64	5.46	
singleplexSample	33679.00	23.12	76.88	21.38	0.00	0.00	
singleplexSample	31657.00	26.07	73.93	13.67	8.62	1.68	
singleplexSample	30740.00	12.01	87.99	9.51	0.00	0.00	
singleplexSample	39035.00	12.92	87.08	5.60	3.98	1.09	
singleplexSample	6047.00	2.91	97.09	0.07	0.00	0.00	
singleplexSample	3388.00	3.16	96.84	0.03	0.00	0.00	
singleplexSample	21694.00	2.53	97.47	0.01	0.00	0.00	

To investigate differences between samples further, go to the **Target Details** page. Here, you can overlay the results of targets from different experimental samples, then export these graphics using the **Export** button in the top right corner. The graphics provided here are the same as when analyzing a single sample, but single samples cannot be overlaid. An example of this is shown below.



A complete video tutorial version of this guide can be viewed on the [rhAmpSeq CRISPR Analysis Tool](#) page.

## Using the rhAmpSeq CRISPR Analysis Tool

Technical support: [applicationsupport@idtdna.com](mailto:applicationsupport@idtdna.com)

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