# Using the rhAmpSeq<sup>™</sup> CRISPR Analysis Tool

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## REVISION HISTORY

Version	Release date	Description of changes
2	June 2023	Updated contact information, account information image, and target report text
1	April 2021	Initital release

#### Table of contents

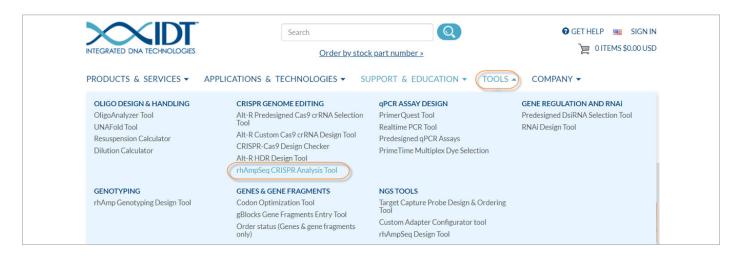
Revision history	2
Introduction	4
Access the rhAmpSeq CRISPR Analysis Tool	4
Create an account	4
Manage your account	6
Start analyzing	7
Upload data	7
Upload large files via Connector setup	9
Data with multiple amplicons	10
Quality control checking BED inputs	11
Handling QC statuses	12
Analyzing singleplex experiments	12
Interpreting singleplex experiments	14
Viewing allele details	15
Viewing the target report	17
Screening guides	18
Analyzing multiplex experiments	18
Interpreting multiplex experiments	20
Viewing allele details	21
Viewing the aggregated results	22

## INTRODUCTION

This analysis guide describes how to use the rhAmpSeq CRISPR Analysis Tool, which was designed for flexible, cloudbased interrogation of CRISPR-mediated genome editing results. This information can also be accessed in video format by viewing the **rhAmpSeq CRISPR Analysis Tool tutorial**.

## ACCESS THE rhAmpSeq CRISPR ANALYSIS TOOL

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



### CREATE AN ACCOUNT

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

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

Login Information		
Login Name:	*	This is the information you will use to log in when you order, view past orders, or change your
Password:	۰	account information.
Confirm Password:	(j) *	
Personal Information		
First Name:		Important:
Last Name:	*	Once established, your first and last name cannot be changed without contacting us;
Email:	*	for organization or institution changes, we ask that you please create a new account
Confirm Email:	*	under the new parent organization. Please contact IDT Customer Care if you have any questions or concerns.
PI First Name:	*	IDT uses your contact information in order
PI Last Name:	*	to provide you with information about our services. IDT does not share your personal
Institution Type:	*	information with any non-affiliated third parties, other than our distributors, nor do we process your personal information for
Institution Name:	*	any other purpose. To learn more about how IDT treats your information, please
I agree to receive email communications from IDT.:	*	review our privacy statement.
I am interested in receiving a free consultation call from IDT.:		

Once established, you will use this account login to sign in and use the rhAmpSeq CRISPR Analysis Tool.

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SIGN IN	REGISTER		

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.

CREATE A LAB
REDEEM CREDIT

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.

CREATE

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Member Name	Invite message				
Gavin Kurgan	Hi, Join my analys	sis lab!		k	
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				SEND	

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

## MANAGE YOUR ACCOUNT

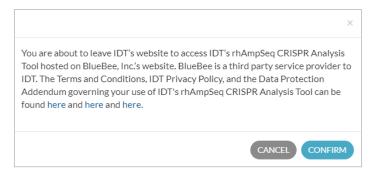
To use the rhAmpSeq CRISPR Analysis Tool, you will need Analysis Credits. You received a code for these credits when the order for your purchased product was invoiced. To redeem your Analysis Credits, click the gray **Redeem Credit** button, then enter the code. View your current balance and transaction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



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## START ANALYZING

Open the rhAmpSeq CRISPR Analysis Tool by clicking the blue Analysis Tool button. Select Confirm when prompted.



A new login page will open where you will enter your IDT account credentials again for security purposes. Now you are ready to begin analyzing data!

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**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.

At any point if you need help or have questions, please **contact us**. We are here to assist you.

### UPLOAD DATA

Before we delve into the process of uploading data, let us review the supported data formats. Specifically, you will have to provide demultiplexed sequencing data, usually in the form of .fastq or .fastq.gz files as shown in the below image. These files contain the following attributes for each read in the sequenced sample:

- read name
- sequence
- associated quality score

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2:		
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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."

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	TestSample-S1_R1 fastq gz (S72 KB)	Os remai

#### Notes:

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

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## 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.

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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

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

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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.

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15       chr.10       106920200       106920200       0114       0       -         16       chr.10       121109396       121109416       OT15       0       -         17       chr.10       125696329       12169941       0       +         18       chr.11       2255645       2525665       OT17       0       +         19       chr.11       2215944       12126040       OT18       0       -         20       chr.11       20102285       20102305       OT19       0       +         21       chr.11       57348913       57348933       OT20       0       -         22       chr.11       64787970       64787990       OT21       0       -         23       chr.11       103863244       0722       0       -         24       chr.12       26976538       0723       0       +         25       chr.12       49647269       0724       0       -         26       chr.12       50808641       0726       0       +         27       chr.12       57289518       0712       0       +         28       chr.12       57289518	13 chr1 206221475 20	5221495 OT12 0	+		
16       chr.10       121109366       121109416       OTIS       0       -         17       chr.10       125506309       125606329       OTIS       0       +         18       chr.11       12252642       2525655       OTIS       0       +         19       chr.11       12125984       12126004       OTIS       0       +         20       chr.11       12125925       2010205       OTIP       0       +         21       chr.11       2010225       2010205       OTIP       0       +         21       chr.11       2010225       2010205       OTIP       0       +         22       chr.11       5038524       10365244       OT22       0       -         23       chr.11       103863244       OT22       0       -         24       chr.12       26976538       26976536       OT25       0       -         25       chr.12       57289518       57289538       OT27       0       +         26       chr.12       57289518       57289538       OT27       0       +         29       chr.12       7484206       7484206       OT28       + </td <td>14 chr10 6437025 643704</td> <td>5 OT13 0 -</td> <td></td> <td></td> <td></td>	14 chr10 6437025 643704	5 OT13 0 -			
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18       chr11       12253645       2525645       0717       0       +         19       chr11       12125984       12126084       0718       0       -         20       chr11       2012285       20102385       0719       0       +         21       chr11       57348933       57348933       0720       0       -         22       chr11       64787990       0721       0       -         23       chr11       103863244       103863244       0722       0       -         24       chr12       26967558       0723       0       -       -         25       chr12       49647289       0724       0       -         26       chr12       57089511       57126       0       +         26       chr12       572895318       57289538       0727       0       +         29       chr12       74842086       7484206       0728       0       +         30       chr12       90948235       90948255       0729       0       -         31       chr12       108046735       108044755       0738       0       -         31	16 chr10 121109396 12	1109416 OT15 0			
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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.

	🕒 Files 🖋 Samples 🔮 Analyses 🔝	Aggregations		≡ ◊ ⊕ ⊠ 0	🔓 IDT customer
INTEGRATED DNA TECHNOLOGIES	Settings				c
	Guide RNA				Save
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	Name	File Name	Size	Creation Date	
	hAlb-1-290f-V4a.bed	hAlb-1-290f-V4a.bed	1.6 KB	2021/01/08 14:21	
	GeneX_guides.bed	GeneX guides bed	3KB	2021/01/04 16:49	

## QUALITY CONTROL CHECKING BED INPUTS

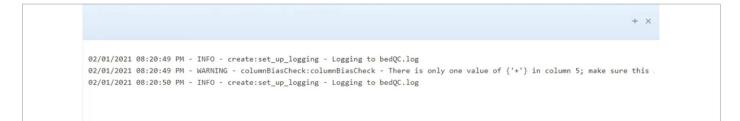
In order to use your BED files for multiplex analysis, the uploaded BED files need to be checked for errors. Navigate to the **Settings** page, then look for the "BED Files Pairs" section. This is where you QC-check your inputs to make sure they can successfully be used for analysis. To begin, select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

▼ BED Files Pairs					0
Pair Name o	Guide RNA 0	Amplicons ÷	Species o	Creation Date	° Status
				₩ ₩	
HBB_guides_dual_min_min	HBB_guides_dual_min_min.bed	HBB_test_amplicons.bed	Homo sapiens - human (GRCh38)	2020/12/04 11:13	Fail
HBB_guides_dual_min_pos	HBB_guides_dual_min_pos.bed	HBB_test_ampliconsFixed.bed	Homo sapiens - human (GRCh38)	2020/12/04 11:57	Pass
HBB_guides_all_dropout_fix	HBB_guides_all_dropout.bed	HBB_amplicons_all_dropout.bed	Homo sapiens - human (GRCh38)	2020/12/04 15:06	Warni
HBB_guides_dual_pos_pos	HBB_guides_dual_pos_pos.bed	HBB_test_ampliconsFixed.bed	Homo sapiens - human (GRCh38)	2020/12/04 14:59	Fail
HBB_guides_dropout_fix	HBB_guides_dropout.bed	HBB_amplicons_dropout.bed	Homo sapiens - human (GRCh38)	2020/12/04 15:08	Warni
HBB_guides_singleplex	HBB_guides_singleplex.bed	HBB_amplicons_singleplex.bed	Homo sapiens - human (GRCh38)	2020/12/04 15:05	Warni
HBB_guides_dual_pos_min	HBB_guides_dual_pos_min.bed	HBB_test_ampliconsFixed.bed	Homo sapiens - human (GRCh38)	2020/12/04 14:58	Pass
HBB_guides_minus	HBB_guides_minus.bed	HBB_test_ampliconsFixed.bed	Homo sapiens - human (GRCh38)	2020/12/04 15:01	Warni
HBB_guides_positives	HBB_guides_positive.bed	HBB_test_ampliconsFixed.bed	Homo sapiens - human (GRCh38)	2020/12/04 15:00	Warni
HBB_guides_dropout	HBB_guides_dropout.bed	HBB_test_ampliconsFixed.bed	Homo sapiens - human (GRCh38)	2020/12/04 15:04	Warni
HBB_guides_multi	HBB_guides_multi.bed	HBB_test_ampliconsFixed.bed	Homo sapiens - human (GRCh38)	2020/12/04 15:03	Warni
HBB_guides_four	HBB_guides_four.bed	HBB_test_ampliconsFixed.bed	Homo sapiens - human (GRCh38)	2020/12/04 14:59	Warni
HBB_guides_dual_min_min_pass	HBB_guides_dual_min_min.bed	HBB_test_ampliconsFixed.bed	Homo sapiens - human (GRCh38)	2020/12/04 11:34	Pass
HBB_guides_all_dropout	HBB_guides_all_dropout.bed	HBB_test_ampliconsFixed.bed	Homo sapiens - human (GRCh38)	2020/12/04 15:04	Fail
DemoPairing	HBB_guides_minus.bed	HBB_amplicons_singleplex.bed	Homo sapiens - human (GRCh38)	2021/02/01 14:34	Ongoi
HBB_guides_positive_1.0.0	HBB_guides_positive.bed	HBB_test_ampliconsFixed.bed	Homo sapiens - human (GRCh38)	2021/02/01 13:57	Warni

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.



## 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.

0

**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 CRISPAltRations software to use for the analysis.

🗋 Files 🥒 Samples 🥹 A	nalyses Lul Aggregations	≣ \$ \$ ⊠ 0	뤎 IDT customer
🖉 Samples	Analyze Singleplex	nalyze Multiplex 🕼 Edit 🕅 Unlink Files	Delete 2
Run Singleplex Analysis		► Analyze	Cancel
	Selected samples (3)		
	Singleplex_GeneX_Control, Singleplex_GeneX_EditedHDR and Single	eplex_GeneX_EditedOnly	
	Analysis		
Analysis mode *	CRISPAItRations 1.0.0	Analysis Tags     Type to add a tag	~

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.

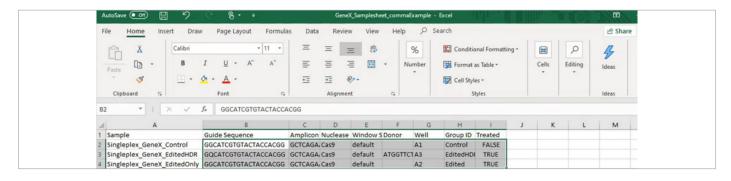
Sample	Guide Sequence	Amplicon Sequence		Nuclease		Window Size		Donor
Singleplex_GeneX_Control	✓		≈	Cas9 🗸	*	default 🐦	*	
Singleplex_GeneX_EditedHDR	×		≈	Cas9 🗸	*	default 🐦	*	
Singleplex_GeneX_EditedOnly	×		]≈	Cas9 🗸	*	default 👽	*	

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

**Tip:** 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.



After specifying your sample information in Microsoft 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.



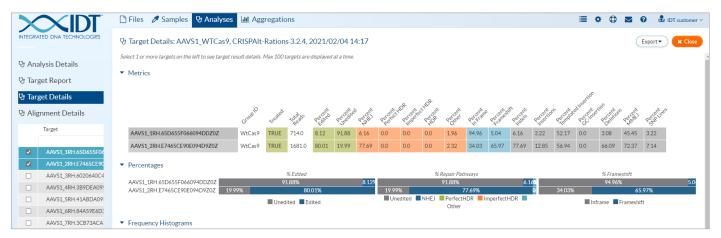
**Caution:** When a red box highlights any of the sample information fields, you will not be able to start the run. Check the hover text on any parameter with a red box to find acceptable inputs and make corrections accordingly. When all inputs are satisfied and no red boxes appear, then you are ready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

XIDT	🗋 Files 🖋 Samples 😲 An	alyses 📠 Aggregations 📰 🌣 🗘 🗹 📽 🖗 💩 IDT customer
INTEGRATED DNA TECHNOLOGIES	8 Samples	► Analyze Singlepice: ► Analyze Multiplex C Edit S Unlink Files Delete
Available analyses CRISPAltRations 50	Run Multiplex Analysis	► Analyze Cancel
_		Selected samples (3)
		AAVS1_Ctrl, AAVS1_HiFiCas9 and AAVS1_WTCas9
		Analysis
	Analysis mode *	CRISPAlt-Rations 3.2.4
	Analysis Tags	Type to add a tag 🗸
		Parameters
		Delimiter*
	Sample BED Files F	Pair Nuclease Window Size Donor
Uploads	AAVS1_Ctrl	K S Cas9 V S default V S S
Downloads No ongoing uploads.	AAVS1_HiFiCas9	BED Files Pair is required
No cloud connector configured for manual import.	AAVS1_WTCas9	v v Cas9 v v default v v v

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 min–2 hr 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.



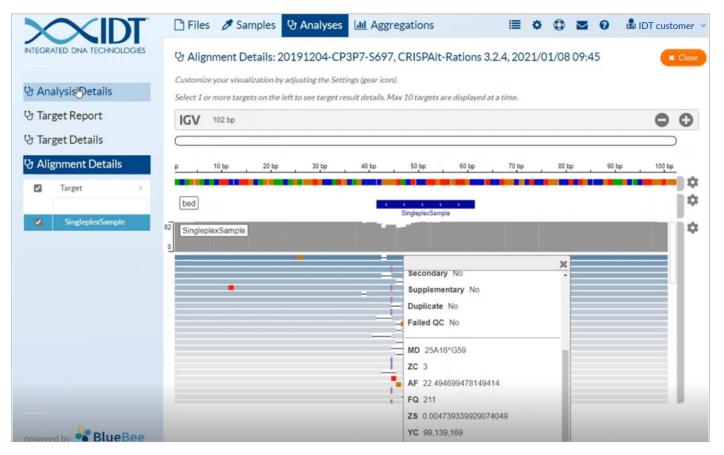
Also, on this page you will be able to find the frequency of the editing, the specific repair pathways used, and any frameshifting events that may have occurred. Dive deeper to explore the exact position of insertion and deletion events in the sequenced sample, even the size of these events. We include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

	Select 1 or more targets on the left to see target result details. Max 100 targets are displayed at a time.
양 Analysis Details	
양 Target Report	✓ Metrics
양 Target Details	a contraction a
안 Alignment Details	
Target o	Edited Edited TRUE 5078.0 97.77 2.23 96.93 0.0 0.0 0.0 0.84 30.03 69.97 96.93 22.98 72.24 1.2 74.99 15.97 8.19
Edited	<ul> <li>Percentages</li> </ul>
	% Edited         % Repair Pathways         % Frameshift           Edited         2.23%         96.93%         30.03%         69.97%
	Unedited Edited Unedited NHEJ PerfectHDR InterfectHDR InterfectHDR InterfectHDR
	<ul> <li>Frequency Histograms</li> </ul>
	Insertions
	Normalized positonal frequency of insertions (out of 100%) for all events annotated as "NHEJ"
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	25 0 37 - 324 - 322 - 30 - 28 - 26 - 24 - 22 - 20 - 18 - 16 - 14 - 12 - 10 - 8 - 6 - 4 - 2 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34
	Amplicon position (relative to cut site)
	د (۱۱) ۲
	- Edited

## 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	Guide Sequence	GGCATCGTGTACTACCACGG	Nuclease	Cas9				~
안 Target Report	A	GCTCAGAATCTGTTCTATGCCCCCAATTTTTCTGTTTCATGCAAGATCTGCCTCC	Max Overlap	152				
양 Target Details	Amplicon Sequence	GCTAAAGTATGACCCCGATGTTGTGGTCACGGATTTCCGCTTTGGGACAATC CCTGTGAAGCTGTACCAACCCAAGGCATCCACCTGCACCCTGAAGCCTGGC	Min Read Size	60				
안 Alignment Details		ATCGTGTACTACCACGGTGGCGGGGGGGCGTCATGGGGAGTTTGAGTAAGAAC CATTTTCTCAGACC	Window Size	default				~
	Donor		Group ID	Edited				
			Treated	TRUE				
	Well	A2	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						
	▼ Results							
	File Name				Size	Format		
	OUTPUT.tar.gz				821.27 KB	UNKNOWN	$\leq$	٥
	SAMPLE_allSnpTables.csv				11.74 KB	CSV	•	٩
	SAMPLE_AllTargetEditing.png				11.69 KB	PNG		(1)
	SAMPLE_collapsedIndeIInfo.csv				305.19 KB	CSV		
	SAMPLE_deleteLocationHist.csv				8.14 KB	CSV		
	SAMPLE_indel.csv				2.96 KB	CSV	9	
	SAMPLE_insertLocationHist.csv				5.62 KB	CSV	9	(1)
	SAMPLE_summary.csv				704 B	CSV		
Se Blue Bee	SUMMARIES.tar.gz				17.94 KB	UNKNOWN	۲	٥
an Turned' company	Results files of the targets are consult	able 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.

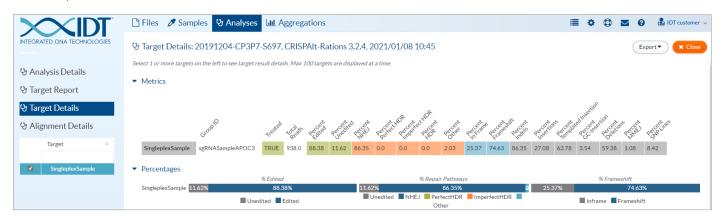
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	69	ACAGGACA	CTTCCT	TGCAGGAACA	GAGGTGCCATGCAG	CCCCGGGTACT	TCCTTG	TGTTGCC	JNK	NOWN (	<b>a</b>
	69				GAGGTGCCATGCAG CGCTCCTGGCCTCTC		CTTGGI	GG	CSV PNG		<b>-</b> (1)
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	43	ACAGGACA	CTTCCT	TGCAGGAACA	GAGGTGCCATGCAG	CCCCGG		,	* CSV	(	•
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## VIEWING THE TARGET REPORT

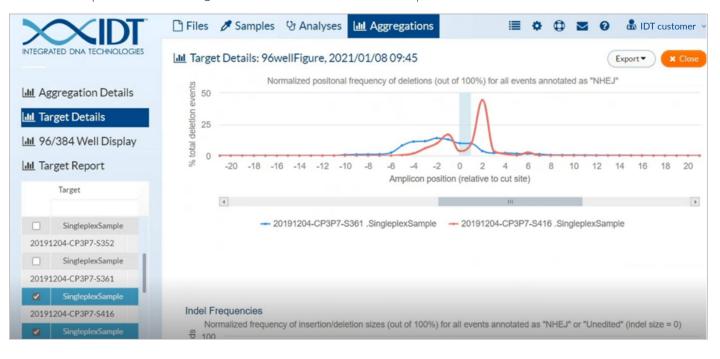
To interpret and compare results of different samples within an experiment, go to the Aggregations tab.

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 different guides, treated under the same conditions. Notice that the indel profiles of the two guides are different, as would be expected.

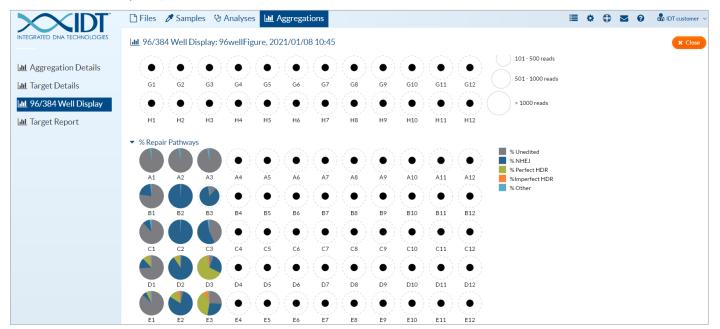


## SCREENING GUIDES

When screening a large number of guides under varying conditions and treatments, you can use the rhAmpSeq Analysis Tool to 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 available for singleplex analyses only. 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.

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0	Samples	► Ana	lyze Singleplex	Analyze	Multiplex	C Edit	S Unlin	k Files 🗎 Del	ete C
Selec	t all samples you wish to an	alyze together.			0				
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		<b>m</b>	<b>m</b>						tutorial
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	AAVS1_WTCas9	2021/02/02 15:	35		New		3.13 MB		Multip
	AAVS1 HiFiCas9	2021/02/02 15:	25		New		2.18 MB		Multip

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 min–2 hr to complete. Your turnaround time will depend on your read depth plus the number of amplicon targets being quantified per sample.

	🗅 File	es 🖉 Samples 😲	Analyses 🛄 A	ggregations	≡ ¢	0 2 0 4	IDT customer
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Available analyses CRISPAItRations 50	Click the	e sample name to view analys Sample	is results of a single sat Progress •	mple. Status =	Reads (total) =	% QC passing 🔹	% Merged
CRISPAltRations 50		20191204-CP3P7-S978		success	4,561	99.76108	99.629875
		20191204-CP3P7-S923		success	33,598	99.65163	99.54672
		20191204-CP3P7-S914		success	10,308	99.50957	99.623116
		20191204-CP3P7-S71		success	6,047	99.523575	99.83493
		20191204-CP3P7-S80		success	21,694	99.619705	99.79303
		20191204-CP3P7-S1259		success	5,841	99.81324	99.64279
		20191204-CP3P7-S135		success	3,389	99.735374	99.97052
		20191204-CP3P7-S416		success	3,398	99.82619	99.44863
		20191204-CP3P7-S352		success	8,849	99.54041	99.71847
		20191204-CP3P7-S361		success	32,353	99.60705	99.71336
		20191204-CP3P7-S642		success	38,877	99.583984	99.64375
		20191204-CP3P7-S1204		success	39,934	99.646835	99.67803
		20191204-CP3P7-S697 20191204-CP3P7-S1195		success	2,275	99.82832	98.79622
		20191204-CP3P7-S1195 20191204-CP3P7-S633		success	4,938	99.53704 99.469284	99.858444 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.

De Analysis Dataila		Select I or more targets on the left to see target result details. Max 100 targets are displayed at a time.
양 Analysis Details		▼ Metrics
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GeneX		▼ Percentages
OT1		% Edited         % Repair Pathways         % Frameshift           GeneX         55.52%         44.48%         55.52%         44.05%         68.09%         31.91%
OT2		Udenek 35.57% 44460% 55.57% 6607% 6607% 51.57%
OT3		
OT4		Frequency Histograms
OT5		Insertions w Normalized positonal frequency of insertions (out of 100%) for all events annotated as "NHEJ"
OT6		Normalized positional frequency of insertions (out of 100%) for all events annotated as "NHE.J"
OT7		
OT8		
OT9		
OT10		2 0
OT11		Amplicon position (relative to cut site)
OT12		1 N N N N N N N N N N N N N N N N N N N
OT13		0.46V

## 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.

양 Ana	lysis Details	Customize your visualization by adjusting the Settings (gear icon). Select 1 or more targets on the left to see target result details. Max 10 targets are displayed at a time.
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	OT6	A CONTRACT OF
	OT7	
	OT8	
	OT9	
	OT10	

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.

앙 Analysis Details	Guide RNA	GeneZZ_guides.bed	₽	Nuclease	Cas9				~
양 Target Report	Amplicons	GeneZZ_amplicons.bed	P	Max Overlap	152				
양 Target Details	Donor			Min Read Size	60				
양 Alignment Details	Well	NotSpecified		Window Size	default				$\sim$
				Group ID					
				Treated	TRUE				
				Species	Homo sapiens - human (GRCh38)				
	Total on-target reads	363,891		% Mapped	100				
	Reads (total)	364000.0		% QC passing	99.99945				
	% Merged	99.970604		% Primer-dimer	0.0				
	% On-target	100.0							
	<ul> <li>Results</li> </ul>								
	File Name					Size	Format		
	OUTPUT.tar.gz					108.8 MB	UNKNOWN	٩	٥
	SAMPLE_allSnpTables.csv					764.44 KB	CSV	P	
	SAMPLE_AllTargetEditing.png					21.41 KB	PNG	₽	٩
	SAMPLE_collapsedIndelInfo.csv					1.29 MB	CSV	P	۷
	SAMPLE_deleteLocationHist.csv					344.52 KB	CSV	₽	٩
	SAMPLE_indel.csv					21.91 KB	CSV	P	۷
	SAMPLE_insertLocationHist.csv					338.14 KB	CSV	P	٤
	SAMPLE_summary.csv					14.29 KB	CSV	P	٩
0.	SUMMARIES.tar.gz					109.36 KB	UNKNOWN	٩	٥
😪 BlueBee	Results files of the targets are consult	able 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.

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		RAG1_GK			2021/01/15 10:2	8 2		ID	T custom	ner			
		TestNonsense			2021/01/15 09:4	4 8		Cu	stomerTe	est			
		CustomerLab			2021/01/14 15:3	7 2		Cu	ıstomerL	ab			
		CustomerNonsens	e		2021/01/08 13:3	2 4		ID	т				
		96wellFigure			2021/01/08 09:4	5 15		96	wellFigur	e			

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.

File Home Insert	Draw Page Layout P	Formulas Dat	ta Review View	Help 🔎 Search	1			1	암 Share 🛛 🖓	Comment
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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.

	268.	•	Target_Report_96wellFigure_2021	_01_08_09_45 (4) - Excel		- /9	
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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.

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A complete video tutorial version of this guide can be viewed on the **rhAmpSeq CRISPR Analysis Tool** page.

#### Using the rhAmpSeq CRISPR Analysis Tool

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

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