

Analyzer Manual

For Research Use Only. Not for use in diagnostic procedures.

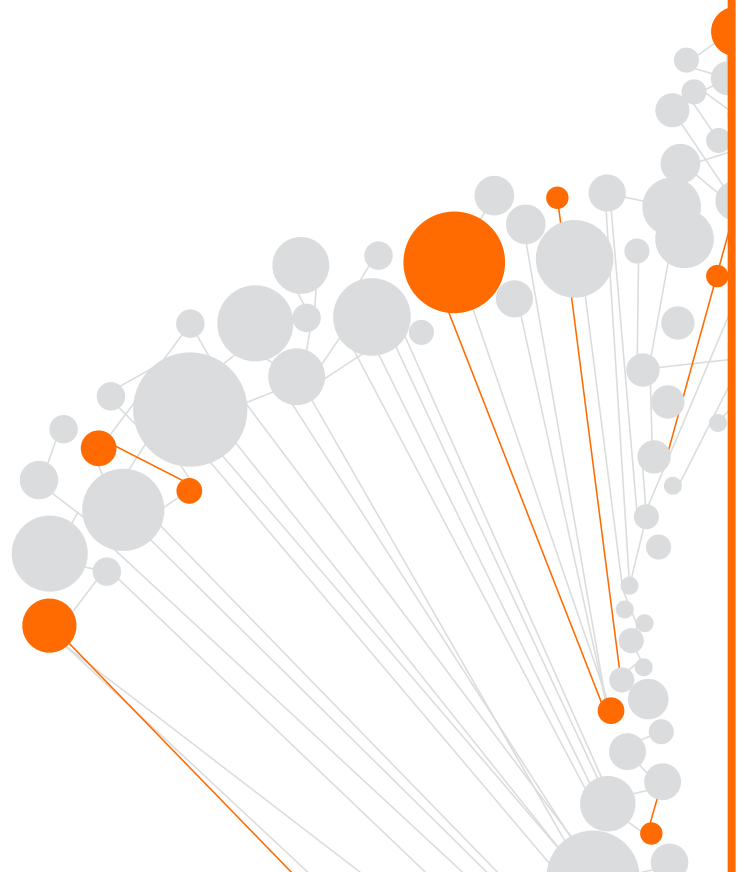


Table of Contents

1. INTRODUCTION	3	3.5 Modifying and Exporting Analysis Figures	29
1.1 What is immunoSEQ?	3	3.5.1 Customize Your Chart	29
1.2 What is the immunoSEQ Analyzer?	3	3.5.2 Download Figure Data	31
		3.5.3 Export a Figure	32
2. LOGGING IN	3	3.6 Viewing Exported Data	32
2.2 Technical Requirements	4	3.7 Editing an analysis	32
2.3 Getting Help	4		
3. DATA ANALYSIS	4	4. MANAGE MY PROJECTS	33
3.1 Selecting an Analysis Tool—Quickstart	5	4.1 Summary Project	33
3.1.1 One-sample Analysis Tools	6	4.2 Creating a New Project	33
3.1.2 Multi-sample Analysis Tools	7	4.3 Sharing a Project	34
3.2 Choosing Samples	7	4.4 Project Details	34
3.2.1 Selecting Individual Samples	8		
3.2.2 Creating and Selecting Sample Groups	8	5. MANAGE MY SAMPLES	34
3.3 Filtering Samples	9	5.1 Basic Sample Details	35
3.3.1 Filter by Project	9	5.3 Sharing a Sample	35
3.3.2 Filter by Tags	10	5.4 Tags	36
3.4 Instructions for Analysis Tools	10	5.5 Editing Tags	36
3.4.1 Gene Frequency	10	5.6 Exporting Sample Information	37
3.4.2 Paired Gene Frequency	12		
3.4.3 V Mark	14	6. SHARED OPEN ACCESS PROJECTS	37
3.4.4 SpectraSEQ	15	6.1 Searching for a Project	38
3.4.5 Clone Tracker	15	6.2 Following a Project	38
3.4.6 Sequence Detail	17	6.3 Share your Project	38
3.4.7 Top Clones	19		
3.4.8 Linear Scatter	20	7. REQUEST A FEATURE	39
3.4.9 Logarithmic Scatter	21		
3.4.10 Heat Map Scatter	21	8. TUTORIAL	39
3.4.11 Common Sequences	22		
3.4.12 Sample Summary	23	9. INDEX	
3.4.13 Sample Export	23		
3.4.14 Explore Edit Distance	25		
3.4.15 Sample Overlap	25		
3.4.16 CDR3 Length	26		
3.4.17 Quality Control	27		
3.4.18 Somatic Hypermutation	28		
3.4.19 QC Report	29		



1. Introduction

1.1 WHAT IS IMMUNOSEQ™?

The immunoSEQ assay is a breakthrough method for measuring the adaptive immune repertoire using high-throughput sequencing of T- and B-cell receptor genes.

The immunoSEQ platform offers a proprietary suite of high-throughput immune profiling assays and powerful online software that allows researchers to characterize the immune repertoire for diverse applications that possess an immunologic component.

immunoSEQ Assay Highlights:

- A highly optimized multiplex PCR-based assay that exclusively targets rearranged T-cell and B-cell receptor genes.
- High-throughput sequencing-based data generation that results in millions of sequences per sample.
- A quantitative description of the adaptive immune cell population ready for further analysis via the immunoSEQ Analyzer.

1.2 WHAT IS THE IMMUNOSEQ ANALYZER?

The immunoSEQ Analyzer is a powerful online software suite that allows the viewing, analysis, and comparison of immunoSEQ data with just a few simple actions. The immunoSEQ Analyzer also readily enables the customization and download of high-quality analysis images suitable for presentation or publication.

immunoSEQ data can be used for many types of studies—case-control studies, time series, tissue vs. periphery comparison, and other analyses—adding valuable insights to an understanding of the immune response in diverse research projects.



2. Logging In

The login page can be reached by visiting <http://clients.adaptivebiotech.com>.

2.1 DEMO SITE

You can use a demo version of the immunoSEQ Analyzer without creating an account. Go to <https://clients.adaptivebiotech.com/immunoSEQDemo> and you will be able to test the majority of immunoSEQ Analyzer's features using sample data sets.

2.2 TECHNICAL REQUIREMENTS

For the immunoSEQ Analyzer, the recommended browsers are Chrome, Firefox, Safari, and Internet Explorer. The “3D Histogram” analysis feature requires a Flash plug-in, but all other analyses will run without additional plug-ins.

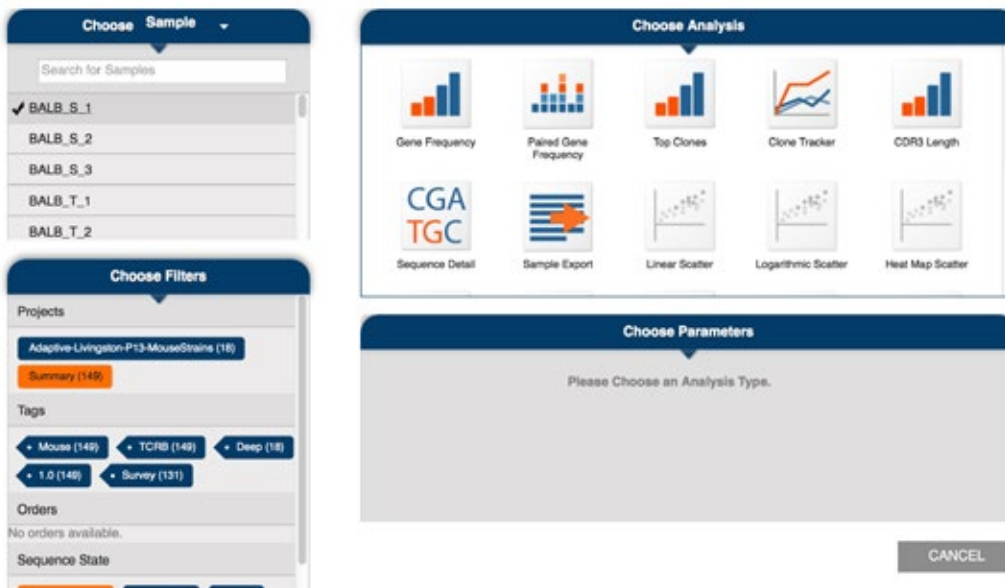
2.3 GETTING HELP

To get help with your account, or with any other aspect of using the ImmunoSEQ Analyzer, contact us via email or phone: +1 (855) 466-8667 (toll free), clientservices@adaptivebiotech.com.

3. Data Analysis

The Analysis feature allows you to examine your data using a variety of computational tools and provides an easy way to make and download graphs and figures of your customized analyses.

To start your analysis, click on the “Analysis” button in the top panel. You will see a dialog box with four panels: Choose Samples, Choose Filters, Choose Analysis, and Choose Parameters (the parameter choices are specific to the analysis you select).



You can see the entire panel by positioning your cursor over the panel; a scroll bar will appear to the right of the choices, enabling and you to scroll up and down.

3.1 SELECTING AN ANALYSIS TOOL—QUICKSTART

The analysis tools in the immunoSEQ Analyzer can help you easily understand and present your data.

If you have a specific table or figure you need to make, please see [Section 3.1.1](#), “One-sample analysis tools,” or [Section 3.1.2](#), “Multi-sample analysis tools” as appropriate. These will point you to the right analysis tool based on the parameters you need to include in your graph or table.

If you need to answer a certain question about your data, but are not sure exactly how to go about it, see the table below. It contains some frequently asked analysis questions and suggested immunoSEQ Analyzer tools which may help you reach your answer.

Frequently Asked Analysis Questions	
How do I...	Suggested immunoSEQ Analyzer Tool(s)
...classify data according to specific VB and J families?	3.4.1 Gene Frequency 3.4.2 Paired Gene Frequency 3.4.6 Sequence Detail
... compare immunoSEQ results to legacy classification methods (FACS and spectratyping)?	3.4.3 VMark 3.4.4 SpectraSEQ
... find the individual clones shared between samples or exclusive to one sample?	3.4.11 Common Sequences
... get a measurement or visualization that reflects the overall degree of clone-sharing between samples?	3.4.15 TCR Overlap 3.4.8 Linear Scatter 3.4.9 Logarithmic Scatter 3.4.10 Heat Map Scatter
... track a specific clone sequence across multiple samples?	3.4.5 Clone Tracker 3.4.11 Common Sequences
... identify the most abundant clones?	3.4.6 Sequence Detail 3.4.7 Top Clones
... look at sequence mutations or the distance between sequences?	3.4.18 Somatic Hypermutation 3.4.14 Explore Edit Distance 3.4.6 Sequence Detail
... check the CDR3 lengths in my samples?	3.4.16 CDR3 Length
... know if I have good sequencing results from my samples?	3.4.12 Sample Summary 3.4.17 Quality Control
... export my sample data in its entirety?	3.4.13 Sample Export

For additional assistance in finding the right analysis tool, please contact us at (855) 466-8667 (toll free) or email clientservices@adaptivebiotech.com.

Once you have selected the appropriate analysis and generated the initial figure, you can modify it to suit your requirements and then export it in a variety of high-quality formats. To learn how to modify the colors, text, axes, and other metrics, see [Section 3.5.1](#), “Customize Your Chart”. To learn how to export, see [Section 3.5.3](#), “Export Figure”.

You can also export the specific data that was used to make the chart. See [Section 3.5.2](#), “Download Figure Data” and then [Section 3.6](#) “Viewing Exported Data”.

3.1.1 ONE-SAMPLE ANALYSIS TOOLS

To find the right analysis tool to make a specific table or figure with data from only one sample, use the table below.

The name of the analysis tool is in blue. The first and second metrics are indicated in the column and row. A third metric is indicated in the boxes, if applicable. Parentheses indicate whether the results are a graphic, a table, or another file type.

Metric 1 \ Metric 2	J gene	% sample, counts	All available data (including frequency, counts, V/D/J annotation, ...)	Total counts, Gene Rearrangements, Unique counts, and Clonality
V gene	Vs.% sample, counts PAIRED GENE FREQUENCY 3.4.2 (graphic)	GENE FREQUENCY 3.4.1 (graphic)		
J gene or V family		GENE FREQUENCY 3.4.1 (graphic)		
TCRB V gene by sequencing or flow cytometry		V MARK 3.4.3 (graphic)		
CDR3 lengths for one TCRBV family (and its V gene members)	# amino acid substitutions EXPLORE EDIT DISTANCE 3.4.14 (graphic)	SPECTRASEQ 3.4.4 (graphic)		
CDR3 lengths for one sample (TCR or IG)		CDR3 LENGTH 3.4.16 (graphic)		
Largest clones (Top N)		TOP CLONES 3.4.7 (graphic)		
Number of V-gene point mutations		SOMATIC HYPERMUTATION 3.4.18 (graphic)		
All clones (by sequences)			SEQUENCE DETAIL 3.4.6 (table) or SAMPLE EXPORT 3.4.13 (.tsv file)	
Sample-level summary			SEQUENCE DETAIL 3.4.6 (top table in browser)	QUALITY CONTROL 3.4.17 (graphic)

3.1.2 MULTI-SAMPLE ANALYSIS TOOLS

To find the right analysis tool to make a specific table or figure with data from two or more samples, use the table below.

Analysis tools for 2 or more samples: The name of the analysis tool is in blue. The first and second metrics are indicated in the column and row. A third metric is indicated in the boxes, if applicable. Parentheses indicate whether the results are a graphic, a table, or another file type.

Metric 2 \ Metric 1	Sample-level stats (including total/unique counts for all sequences and just productives, entropy, clonality, max frequency)	Total counts, Gene Rearrangements, Unique counts, and Clonality	Sample names	Counts or % total	Counts or % Sample 2	All information
Sample names	SAMPLE SUMMARY 3.4.12 (<i>table</i>)	QUALITY CONTROL 3.4.17 (<i>graphic</i>)	Overlap and Pearson correlation TCR OVERLAP 3.4.15 (<i>graphic</i>)			
All clones or overlapping clones				COMMON SEQUENCES 3.4.11 (<i>table</i>)		
Selected clones				CLONE TRACKER 3.4.5 (<i>graphic</i>)		
V Gene/ family or J gene,				GENE FREQUENCY 3.4.1 (<i>graphic</i>)		
CDR3 length				CDR3 LENGTH 3.4.16 (<i>graphic</i>)		
Number of V-gene point mutations				SOMATIC HYPERMUTATION 3.4.18 (<i>graphic</i>)		
Counts or % Sample 1					2 samples only— All overlapping sequences LINEAR SCATTER 3.4.8, LOGARITHIC SCATTER 3.4.9, HEAT MAP SCATTER 3.4.10 (<i>graphic</i>)	
All clones, all samples						SAMPLE EXPORT 3.4.13 (<i>.tsv file</i>)

3.2 CHOOSING SAMPLES

To start your analysis, click on the “Analysis” button and select the samples or sample group(s) you wish to analyze.

3.2.1 SELECTING INDIVIDUAL SAMPLES

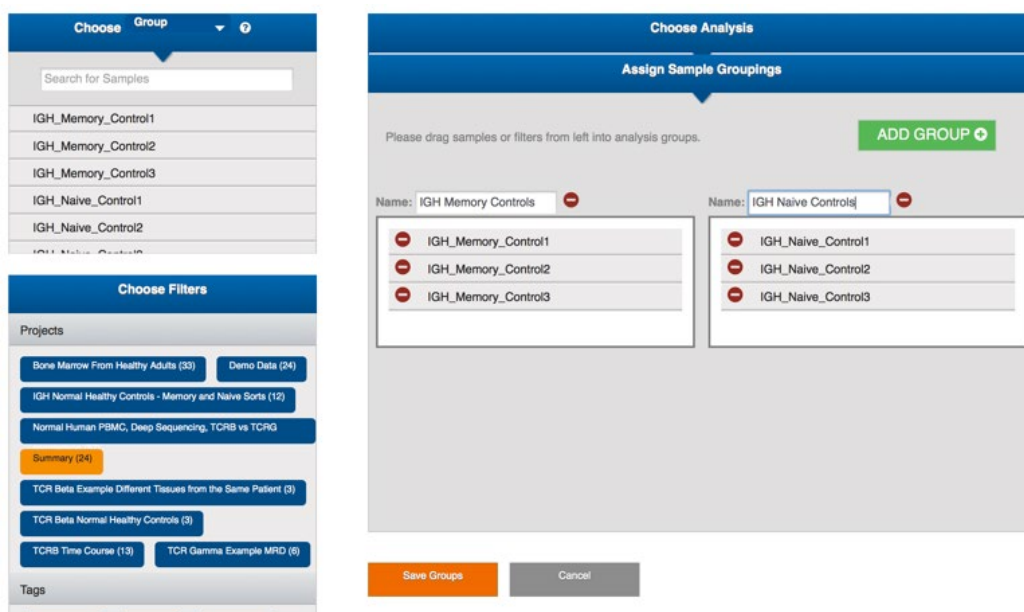
In the “Choose Sample” panel, click on each sample you want to include in your analysis. Click each independently or select a block of samples. To select a block of samples on a Mac, click on the first sample, then hold down command-shift at the same time and click on the last sample. To select a block of samples on a PC, click on the first sample and while holding down the CTRL button, click any other samples you wish to include.

You can use the search box to find specific sample sequences matching a given string in the name. If the list is too long to easily select the samples you want, you can create a project containing a subset of the samples (“Managing My Projects”). Then, you can use the “Choose Filters” feature to make your list more manageable (See [Section 3.3.1, “Filter by Project”](#)).

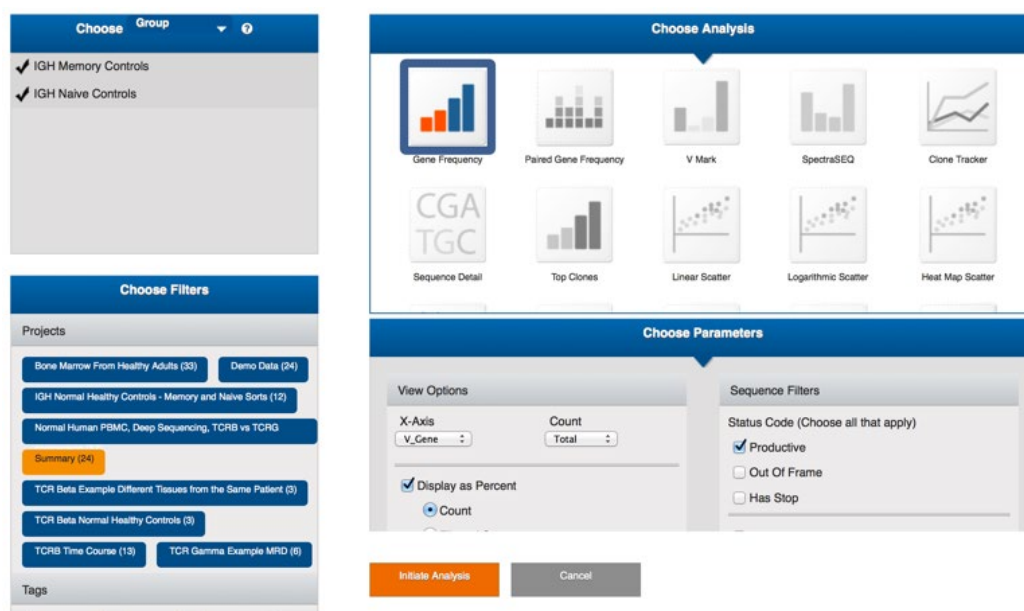
3.2.2 CREATING AND SELECTING SAMPLE GROUPS

If you want to compare groups of samples, you can use the sample groupings feature. In the upper left “Choose Sample” analysis panel, click on the drop-down menu labeled “Sample” and select “Group”. If you have not yet saved any groups, an “Assign Sample Groupings” panel will appear on the right. You must assign at least two groups.

To assign a group, drag the sample names from the list of samples into each of the sample boxes one at a time. Or, drag a filter, tag, or order into the box and it will auto-populate with the members of that group. Add a unique name for the group at the top of each sample box.



To get a new box for an additional group, click the “Add Group” button at the upper right. When you are done building your groups, click the “Save Groups” button below the panel.



Once you have groups saved, you may again use the drop-down menu to choose groups for analysis. Currently, “Gene Frequency” is the analysis enabled for groups. In the resulting graph, you will see that for each category in the histogram, there is one entry per group.

3.3 FILTERING SAMPLES

Filters can be used to reduce the length of the list of potential samples. You can filter the sample list using either projects or tags, or both. To customize the filters to your needs, you can modify tags using the “Samples” page and modify projects using the “Projects” page.

3.3.1 FILTER BY PROJECT

By default, the “Summary” project will be selected. “Summary” is the full list of all your available samples. To deselect “Summary,” simply click on it. To choose a different project, click on its name. You may select multiple projects at a time and all samples in the selected projects will appear in your sample list.

The only samples that will not be in the Summary folder are samples belonging to a public project you are following (“Shared Open Access Projects”). For example, if you choose to follow the “TCRB Time Course” project, a “TCRB Time Course” button will appear in your “Choose Filters” box. You will see those samples only when you select the “TCRB Time Course” project. If you would like to compare the data from the public TCRB Time Course project to your own data, you may select both the TCRB Time Course and the Summary filters.

Similarly, if a colleague has shared a project with you, a custom filter for that project will appear in your filter list, enabling you to selectively view or hide those samples from the list.

3.3.2 FILTER BY TAGS

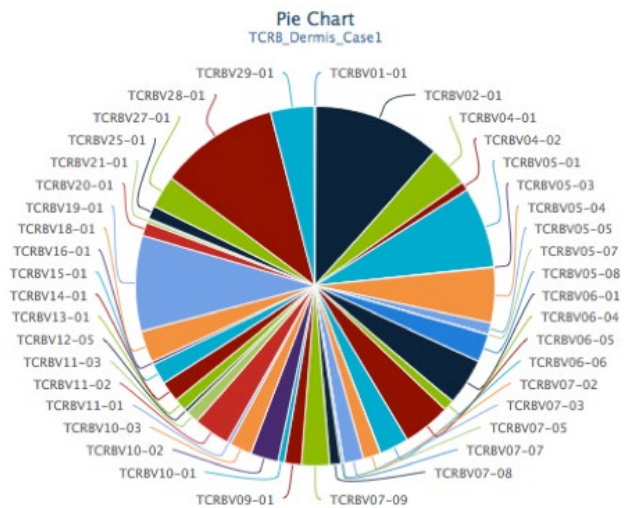
If you would like only samples with certain characteristics to be in your sample list for analysis, you can use the tags function. If you choose a tag (i.e., IGH), only samples tagged with “IGH” will appear in your sample list. If you select more than one tag, only samples that have all selected tags will be shown. For example, if you select the “IGH” tag and the “Deep” tag, you will only see those samples that have been tagged with both “IGH” and “Deep.”

3.4 INSTRUCTIONS FOR ANALYSIS TOOLS

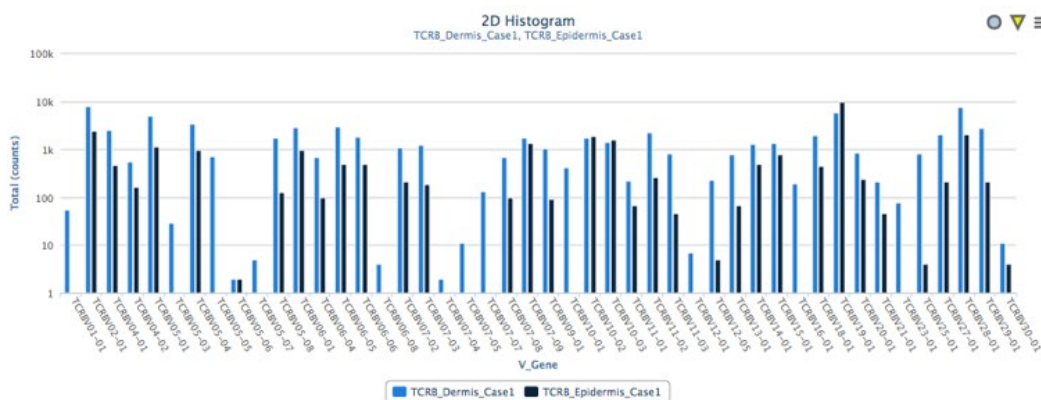
Many different analyses are available. Before you select a sample, all of the “Choose Analysis” options will be grayed out. When you select one sample, the analyses that can apply to that sample appear in color and may be selected. When you select a second sample, only analyses applicable to the two samples appear in color and may be selected. When you select three or more samples, only analyses applicable to greater than two samples appear in color and may be selected.

3.4.1 GENE FREQUENCY

The Gene Frequency analysis is available for any number of samples, for any locus. It allows you to view the V or J breakdown of each sample. For a single sample, you can view the results as either a pie chart or as a 2D histogram.



For two or more samples, you may view the results only in a 2D histogram. The x-axis lists each observed member of the selected family/gene (either V families, V genes, or J genes, depending on what option is selected). The bar height indicates the proportion of that gene in the sample, either by read count or frequency. Alternatively, you can choose to view the histogram by gene family or broken down further by allele.



To run this analysis, select your sample(s) in the “Choose Samples” box and click on the “Gene Frequency” icon in the “Choose Analysis” box. A parameter dialog box will appear in the lower right.

The following parameters will be available to select:

X-Axis: V Gene, J Gene, or V Family.

Count: Total, Median, Average, or Unique.

Display as Percent: By default this box is checked and the gene or family amounts are displayed as a percentage of total counts for that sample. There is also a “Filtered Sequences” option to remove partial DJ rearrangements in IGH data.

Use Logarithmic Scale: If checked, the y-axis will be logarithmic.

Include Unresolved: If checked, sequences for which V or J could not be resolved are included in a separate area marked “Unresolved.”

Visualization Type: 2D Histogram or Pie (only available for single samples).

Status Code: (more than one may be chosen): Productive, Out Of Frame, and/or Has Stop (see glossary for detailed description).

Include Alleles in Query: This makes the match more stringent. Both the gene and alleles must match.

Filter DJ Sequences: Relevant for IGH samples. By default, DJ rearrangements are filtered out, leaving only VDJ rearrangements. Uncheck this box to include DJ rearrangements.

When you have selected your parameters, click the orange “Initiate Analysis” button at the bottom.

If you are viewing the single-sample results as a pie chart, hover your cursor over each of the pie slices, or over each of the gene/family labels, and a box will pop up detailing the count for each section. Download the chart data and you will get a table listing each of the families and count (or percentage) for each family.

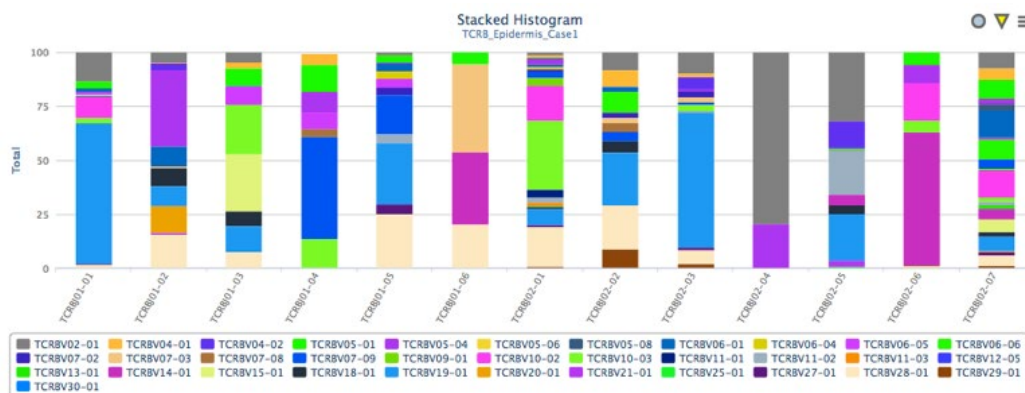
If you are viewing the results as a 2D histogram, hover your cursor over any of the bars, and a box will pop up detailing the count (or percentage) for that bar.

To modify or export the resulting histogram, see [Section 3.5, “Modifying and Exporting Analysis Figures.”](#)

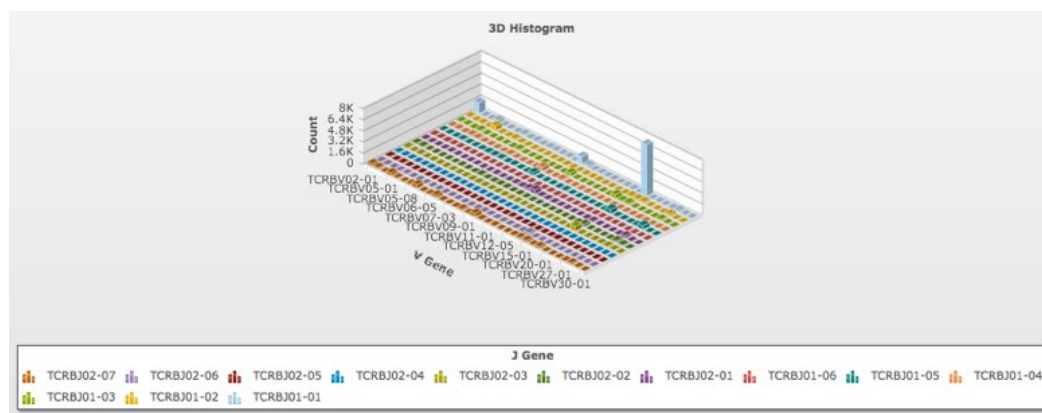
3.4.2 PAIRED GENE FREQUENCY

The Paired Gene Frequency analysis is available for single samples sequenced on any locus. For the single sample selected, it gives you a visualization of the V-J combinations included in that sample. You can view them as a stacked histogram, a 3D histogram, or a series of donut plots (examples below).

Stacked Histogram:



3D Histogram:



Donut plots:



To run this analysis, select a single sample in the “Choose Samples” box. Then click the “Paired Gene Frequency” icon in the “Choose Analysis” box. A “Choose Parameters” dialog box will appear. There are View Options and Sequence Filters.

The following parameters will be available to select:

Count: Total, Median, Average, or Unique.

Include Unresolved: If checked, sequences for which V or J could not be resolved are included in a separate area marked “Unresolved.”

Visualization Type: Stacked Histogram, 3D Histogram, or Donut.

Status Code: Productive, Out Of Frame, and/or Has Stop (see glossary for detailed description).

Include Alleles in Query: This makes the match more stringent. Both the gene and alleles must match.

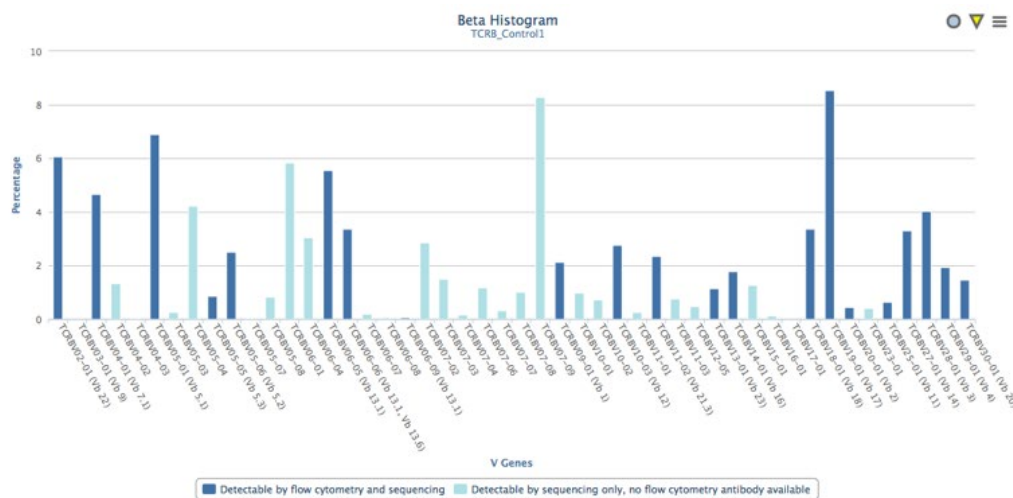
Filter DJ Sequences: Relevant for IGH samples. By default, DJ rearrangements are filtered out, leaving only VDJ rearrangements. Uncheck this box to include DJ rearrangements.

In all three plot types, you can hover your cursor over any of the segments and a box detailing the counts will appear.

3.4.3 V MARK

The V Mark analysis is a single-sample analysis specific to human TCRB samples. It gives you the information necessary to compare your immunoSEQ TCRB results to available flow cytometry data for the same sample.

For each sample, the V Mark analysis returns a histogram bar graph. The x-axis is each TCRB V gene, and the y-axis is the percentage of the sample that falls into that category. Bars that are colored dark blue are detectable by both flow cytometry and sequencing; bars that are colored light blue are TCRB genes lacking a flow cytometry antibody and detectable by sequencing only. The x-axis labels that have flow cytometry Vb equivalents are marked as such in parentheses. By looking only at the dark bars, you can compare your sequencing results to flow cytometry results.



To run this analysis, select your TCRB sample from the “Choose Samples” panel at the left. Then select the “V Mark” analysis by clicking on the icon in the “Choose Analysis” box.

The only parameter available to choose is “Filter DJ Sequences.” This is automatically selected, which means that only VDJ sequences are included and DJ sequences are not included. To include the DJ sequences, uncheck the box.

Click the orange “Initiate Analysis” button at the bottom of the page to run the analysis.

If you would like to create a bar graph using only the betas with flow cytometric equivalents, download the data via the yellow inverted triangle to the top right of the graph. The TCRB V segments with flow cytometric equivalents are listed first so that you can easily select only those for your analysis. The first column contains the TCRBVs. The second column contains the total counts observed for each TCRBV, and the third column indicates the percentage of the total counts (for all TCRBVs combined) that the individual TCRBV represents.

See [Section 3.5, “Modifying and Exporting Analysis Figures”](#) for more details.

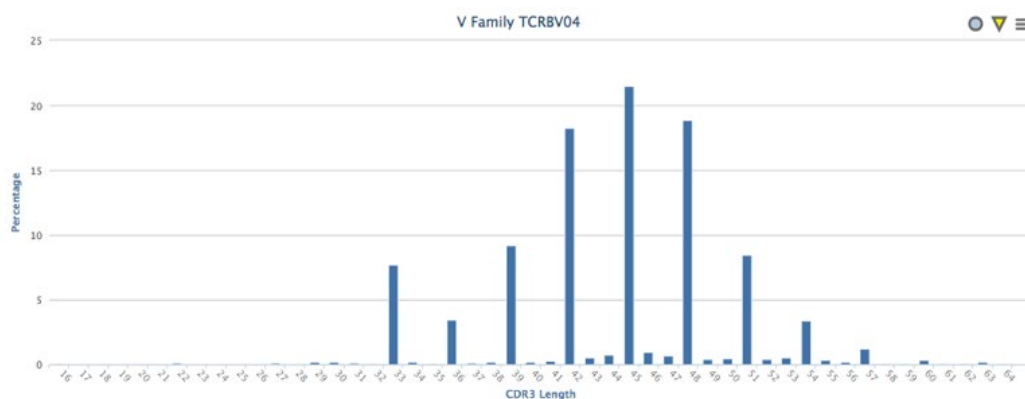
3.4.4 SPECTRASEQ

SpectraSEQ is a single-sample analysis specific to human TCRB samples. This analysis gives a histogram of CDR3 length vs. percentage for whichever TCRB family you select, both at the family level and for genes in that family corresponding to specific antibodies. If you would like a CDR3-length histogram for the entire sample, or for samples other than human TCRB, use the “CDR3 Length” analysis option instead of the “SpectraSEQ” analysis option.

To run the SpectraSEQ analysis, select your human TCRB sample from the “Choose Samples” panel at the left. Then select the “SpectraSEQ” analysis by clicking on the icon in the “Choose Analysis” panel.

There are two parameters available to select: the V family and “Filter DJ Sequences.” “Filter DJ Sequences” is automatically selected, which means that only VDJ sequences are included and DJ sequences are not included. To include the DJ sequences, uncheck the box. After selecting a V family and DJ sequence filter, click the orange “Initiate Analysis” button at the bottom of the page.

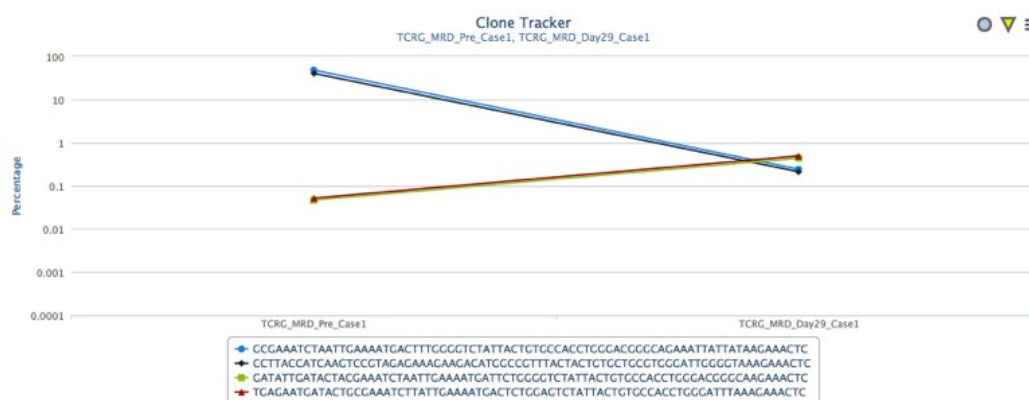
You will see a series of histograms. The first will be for the entire V family. This includes all the members of the V family with and without antibodies available. The subsequent histograms are for the individual members of the family. On each histogram, it is indicated whether there is an antibody available or not. If there is no antibody available, that means that the information is available only via sequencing.



The x-axis of the histogram indicates the CDR3 length (in nucleotides) and the y-axis indicates the percentage of reads in the sample with that CDR3 length. You can download the histograms themselves or the underlying data via the icons at the upper right corner of the figure. See [Section 3.5, “Modifying and Exporting Analysis Figures.”](#)

3.4.5 CLONE TRACKER

The Clone Tracker is available for all loci and for any number of samples. It allows you to view the frequency of a number of clones across multiple samples. You can view this information either as a line plot or as a table. If viewed as a line plot, the x-axis will contain each of the selected samples in the order you chose them. The frequency of the top clones in each sample will be tracked across all of the selected samples.



If the results are viewed as tabular data, you will see a table containing the top clones of each sample and their frequency in the other samples.

Nucleotide	Total	TCRG_MRD_Day29_Case1 frequency	TCRG_MRD_Pre_Case1 frequency
GCGAAATCTAATTGAAATGACTTTGGGCTCTATTACTGTGCCACCTGGGACGGGAGAAATTATATAAGAACTC	209814	0.24129852074137434 %	47.88315357432084 %
CCTTACCATCAAGTCGTAGAGAAAGACATGGCGTTTACTACTGTGCTGGTGGGTAAGAACTC	175344	0.21026012180783654 %	40.0026687771479 %
TGGAATGATAGCGAAATCTATTGAAATGACTCTGGAGTCTATTACTGTGCCACCTGGGATTTAAGAACTC	3664	0.4918942577531632 %	0.05176507388954575 %
GATATTGATAGTACGAAATCTAATTGAAATGATTCTGGGCTCTATTACTGTGCCACCTGGGACGGGCAAGAACTC	3266	0.4381134374812268 %	0.04670360088345724 %

To run the Clone Tracker analysis, select your samples in the “Choose Samples” box and click the “Clone Tracker” icon in the “Choose Analysis” panel. In the “Choose Parameters” box, you will see four tabs with customization options: Samples, Sequences, Visualization Options, and Compare Options.

Samples: In this tab, you can select what samples you want to include, as well as the order in which the samples will appear. You can use the “Type Sample or Tag Name” input box to filter the selection list. The samples you selected in the “Choose Samples” panel will be automatically selected, but you can add or remove samples in this tab by selecting or de-selecting a sample by clicking on the sample name. To order the samples, use the yellow button with the up/down arrows to the left of each sample name. Hover your mouse over the yellow button, click and hold the mouse button down to grab the sample, and drag the sample up or down to the appropriate location. Release the mouse button to “drop” the sample name into place.

Sequences: This allows you to set a parameter to select what sequences you will view. If you have selected more than one sample, you can either select or enter the number of top clones to view (the default number is 10), or select to view clones greater than a set percentage of the sample (the default percentage is 5%). There is also a place to select tags if you wish to filter sequences based on individually applied sequence tags.

If you select only one sample, the “Sequences” parameter tag lists all of the sequences. You may select what sequences to view by clicking on the individual sequences.

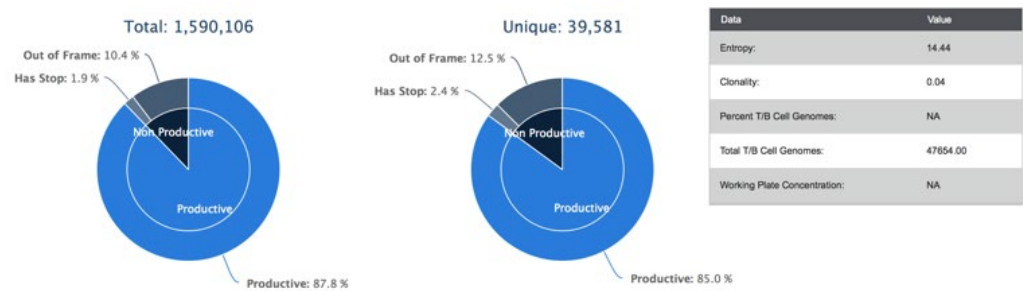
Visualization Options: You can view the top clones as either tabular data or as a line plot. If you choose to view a line plot, you also will have the option to view the y-axis in logarithmic

scale. You can choose to display the copy counts in the y-axis as the percent of total reads or as raw counts. If you choose to view the data as a table, you may export the data in .zip form by clicking the dark blue “Export” button at the upper left of the table.

Compare Options: This allows you to compare clones between samples based on Nucleotide, AminoAcidVJ, or AminoAcid. If “Nucleotide” is selected, by default the entire DNA sequence must match, but you can also pick the number of base pairs to match. Matching begins at the first position in the sequence and extends out to the number of base pairs specified by this parameter. If “AminoAcidVJ” is selected, both the amino acid sequence and the V and J segment must match, whereas if “AminoAcid” is selected, only the CDR3 amino acid sequence is considered for matching purposes. You also can choose whether to include DJ sequences in addition to VDJ sequences (by default they are filtered out).

3.4.6 SEQUENCE DETAIL

The Sequence Detail analysis is available for any single sample. Select your sample in the “Choose Samples” box. Then select the “Sequence Detail” icon in the “Choose Analysis” box. You can pick whether to view the results as pie charts or as a table, then select whether to include only VDJ sequences (default) or to include DJ sequences as well. Uncheck the “Filter DJ Sequences” box to include the DJ sequences. Then click the orange “Initiate Analysis” button at the bottom of the page.



If you chose to view the results as pie charts, you will see two pie charts at the top of the page, one with the total number of counts and one with the number of unique counts. The percentage of each group that is productive vs. nonproductive will be indicated, and the nonproductive will be broken down further into “Out of Frame” and “Has Stop.” If you hover your mouse over the percentage, it will indicate the count for each section. Choosing to view the results as a table, you will see the percentages of productive and nonproductive sequences for both total and unique sequences.

On the upper right, you will see a small table with values for Clonality, Gene Rearrangements, Productive Gene Rearrangements, and Mass of Reaction Template (ng), if available. The entropy reported is Shannon’s entropy of the distribution of read counts for each TCR or BCR sequenced. This is a measure of the shape of that distribution and includes information about the number of TCR sequences recovered (more sequences leads to a higher entropy) and how clonal the sample is (highly clonal samples have low entropy; very diverse samples have high entropy). Clonality is defined as (1-normalized entropy), where normalized entropy is entropy/ log₂(productive_uniques). Clonality ranges from 0 to 1. A low clonality number indicates a

relatively diverse sample, while a high clonality number indicates that the sample is dominated by a few clones, or possibly even a single high-frequency clone.

Sequence Detail: IGH_Memory_Control2

Summary Statistics

Filters	Sequences	% of Total Sequences	Unique Sequences	% of Total Unique Sequences
Total	1,590,106		39,581	
Productive	1,395,784	87.78%	33,661	85.04%
Non Productive	194,322	12.22%	5,920	14.96%

Data	Value
Entropy:	14.44
Clonality:	0.04
Percent T/B Cell Genomes:	NA
Total T/B Cell Genomes:	47654.00
Working Plate Concentration:	NA

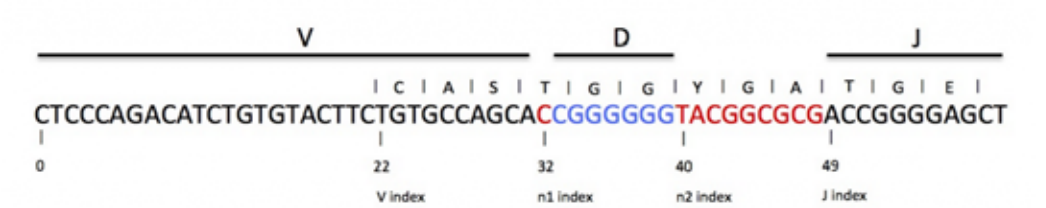
Clone Tracker Tag Sequence Columns Filters Show 10 entries Search: Count 1100

Frequency	Count	V	D	J	Amino Acid	Nucleotide
0.3473%	5,522	IGHV03-66	IGHD03-10	IGHJ05-01*02	CASVTGGDYDFPW	TGTGCGAGTGTAAACAGGCGGGACTATTTCGACCCCTGG
0.2302%	3,661	IGHV03	IGHD05	IGHJ04-01*02	CASPQEDQLLYW	TGTGCGAGCCCCAAGAGGATATACAGCTCCTAGGCTACTGG
0.0924%	1,469	IGHV03	IGHD03-03*01	IGHJ06-01*02		TGTGCGAAAGCGCGCGGGGCTACGGATTACGATTTTGGAGTGAGTGGTGAATTCCTACTAGGATTATP
0.0851%	1,353	IGHV06-01	IGHD04	IGHJ01-01*01	CARGIPRSWSDYSDFNHW	TGTGCAAGAGGGATCCCGAAGCTGGTCTGACTACAGTGACTATTTCATCACTGG
0.0711%	1,130	IGHV03-72	IGHD01-26*01	IGHJ06-01*02		TGTGCTAGAGCCCTAGTGGAGCCACACCTCGGCTCCTACTACTACGGTATGGAGCTCTGG

Showing 1 to 5 of 5 entries (filtered from 39,671 total entries) First Previous 1 Next Last

On the lower half of the page, you will see a table of all of the clones contained in the sample. Columns included by default are Frequency, Count, V, D, J, Amino Acid, Nucleotide, Rearrangement, Fraction Nucleated, Estimated Number of Genomes, and Somatic Hypermutation of V Gene (for IGH or IGKL samples). To see additional columns, position your cursor over the table and scroll to the right. A scroll bar is located at the bottom of the table. You can sort by any of the columns by clicking on the column header. Click once to forward-sort and click again to reverse-sort.

In the Nucleotide column, sequences are annotated with four colors: black, red, light blue, and dark blue. Reading from the left, black indicates the V gene, red indicates the non-templated insertions of the random N1 region, light blue indicates the 5' germline sequence of a DJ rearrangement, and dark blue indicates the D gene. The next red nucleotides are the non-templated insertions of the N2 region and the next black nucleotides are the J gene. In the Somatic Hypermutation of V Gene column, mutated bases are colored in red. There are many other features of the data that can be viewed as well, and you can add or subtract these columns using the dark blue "Columns" button above the table and checking or unchecking the columns you wish to view.



On the upper right of the table, there is a search feature where you can build a regular expression search. You can select any of the available columns to search via the pull-down menu, and an appropriate search criterion using the following options:

Contains	(for text-based columns)
Not Contains	(for text-based columns)
=	(equals, for an exact match for either text or numeric columns)
!=	(not equals, to exclude an exact match for either text or numeric columns)
>	(greater than, numeric)
>=	(greater than or equal to, numeric)
<	(less than, numeric)
<=	(less than or equal to, numeric)

You can type in your term of interest in the blank box after the search criteria, and click the magnifying glass icon to perform the search. The search results will be reflected in the table. If you then perform an additional search, your second search will be applied to the results of the first search.

Once you have performed a search, you will see a dark blue “Filters” button on the upper left of the table. This lists all of your search terms that are active. You can remove a search term by unchecking the box next to the term. The table will automatically update.

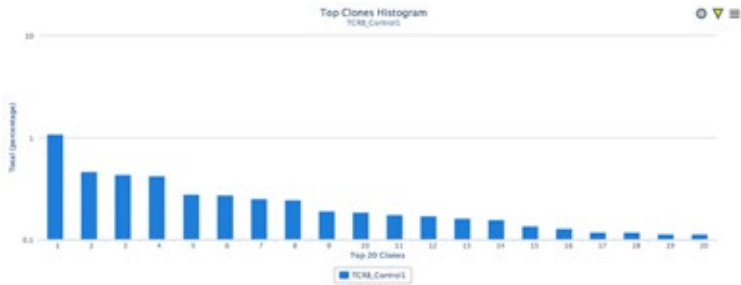
The dark blue “Tag Sequence” button to the upper left of the table allows you to individually tag specific sequences associated with a clone of interest. These sequence-level tags can then be used at a later time to identify the clone in your “Clone Tracker” analysis tool.

If you would like to track specific sequences of interest you find in this analysis, highlight the sequences by clicking on each one. You can then click the “Clone Tracker” button above the table and it will bring you directly to the Clone Tracker parameters box, with your chosen sequences already selected. See [Section 3.4.5, “Clone Tracker.”](#)

Above the table, there is a pull-down menu that allows you to adjust the number of table entries visible. At the bottom of the table, you will see how many total entries there are and what entries you are looking at. Use the buttons at the bottom of the table to page through the results.

3.4.7 TOP CLONES

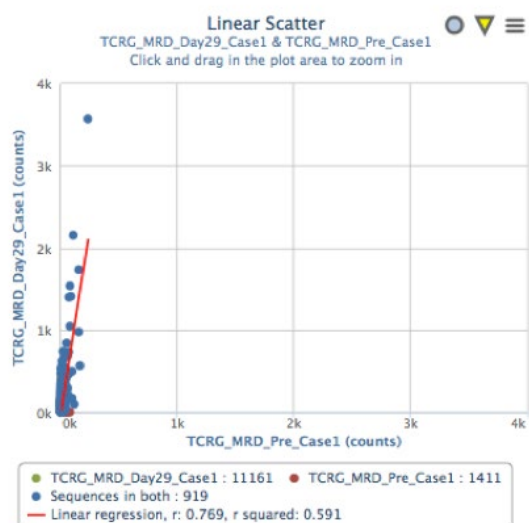
The Top Clones feature is available for any single sample. It returns a histogram of a given number of top clones based on the percentage of sequencing reads represented by each clone in the sample.



To run this analysis, select a sample and then click the “Top Clones” icon. You will have several options in the “Choose Parameters” box. First, you can enter the number of top clones you wish to view. Next, choose whether to display the y-axis as total counts or a percentage and whether to use a logarithmic scale. You can choose to include unresolved clones where either the V or J gene is unable to be conclusively determined. You also can filter what sequences to include. By default, only productive sequences are included, but you can choose to include “Out Of Frame” sequences, or “Has Stop” sequences. Check the box for “Include Alleles in Query” to make the matching more stringent. By default, DJ sequences are filtered out, and only VDJ sequences are viewed. Click the “Filter DJ Sequences” box to include DJ sequences.

3.4.8 LINEAR SCATTER

Linear Scatter analysis is available for any two samples. This is a scatter plot with a sample on each axis. Each clone is plotted according to its quantity in the two samples.



To run this analysis, first select two samples from the “Choose Samples” box and then click the “Linear Scatter” icon in the “Choose Analysis” box. You have three choices on which to compare the clones in the samples: “Nucleotide”, “Amino Acid V, J” (amino acid clones that also use the same V and J genes to produce the CDR3), and “Amino Acid Only”. For “Nucleotide,” you can select the number of base pairs to match; by default this is the entire length. While “Amino Acid Only” matches only on the CDR3 sequence, “Amino Acid V, J” also matches on the V and J alleles. You can use match specificity for this choice to match at the V family level or the V gene level.

The data can be displayed on the x- and y-axes as percentages (by clicking “Display as Percent”) or as counts if that box is unchecked. You may also choose to compute the Pearson coefficient between the two samples.

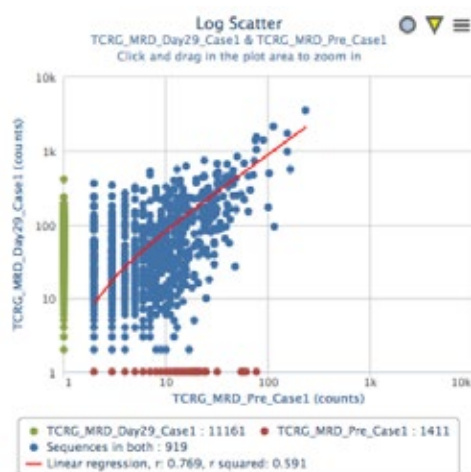
As a default, only “Productive” sequences will be included, but you also can choose to include “Out Of Frame” and “Has Stop” sequences as well. “Include Alleles in Query” can be used to make the match more stringent. Additionally, you may filter out (default) or include DJ sequences.

Once you view the scatter plot, you may use your cursor to zoom into a particular section by clicking it in the corner of the area you want to look at and dragging a box around the area of interest. Let go of the cursor and the plot will automatically zoom in. After you have zoomed in, there is a “Reset zoom” button in the upper right corner of the scatter plot. Click it to return to your original view.

If you hover your mouse over any point, a box will pop up showing the sequence that point represents and the counts for that sequence for each sample. You may also click on any data series in the legend to remove it from the graph or to add it back in once removed.

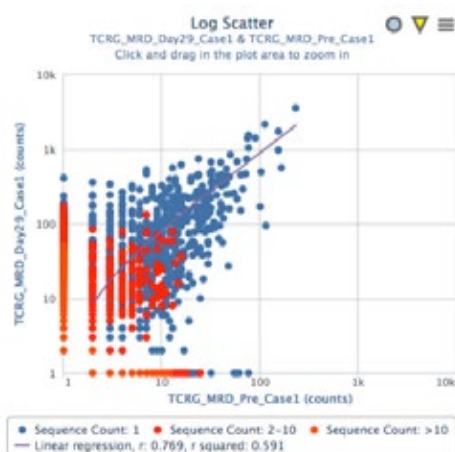
3.4.9 LOGARITHMIC SCATTER

The Logarithmic Scatter analysis is identical to the Linear Scatter analysis, except that in the Logarithmic Scatter Analysis, the x- and y-axes are on a \log_{10} scale. See [Section 3.4.8, “Linear Scatter.”](#)



3.4.10 HEAT MAP SCATTER

Heat Map Scatter is identical to Logarithmic Scatter, except that the dots in the Heat Map Scatter are colored based on how many unique sequences overlap at the same location on the X-Y axis. See [Section 3.4.8, “Linear Scatter”](#) and [Section 3.4.9 “Logarithmic Scatter”](#).



3.4.11 COMMON SEQUENCES

The Common Sequences analysis is available for two or more samples of any locus. It returns either a union table or an intersection table for sequences in the selected samples. To run this analysis, first select the samples you wish to compare from the “Choose Samples” box and click the “Common Sequences” icon in the “Choose Analysis” box. The following parameters are available to select:

Compare Using: “Nucleotide”, “Amino Acid V, J” (for identical V and J genes), or “Amino Acid Only”.

Base Pairs to Match: Entire length vs. an input amount (if using Nucleotide to compare).

Match Specificity: V Gene or V Family (if using Amino Acid, V, J to compare).

Display as Percent: By default the levels of the clones are displayed in counts, but you can check this box to display the levels of the clones as a percentage of the sample.

Status Code: Productive, Out Of Frame, Has Stop (more than one may be chosen).

Set Operation: Intersection or Union. If you select Intersection, you will see only those sequences that were found in every sample you selected. If you select Union, you will see a table with all the sequences found in one or more samples, with count entries for the sequence in each sample as well as the “union” of all samples.

Number of Top Clones: Instead of comparing all clones, this option allows you to look at only the top N clones in forming an intersection or union. A sequence only needs to be in the Top N in one of the samples to be present in the intersection or union table.

Include Alleles in Query: This makes the match more stringent. Both the gene and alleles must match.

Filter DJ Sequences: By default this is checked, which means you only view VDJ rearrangements. If you uncheck the box, you will also see DJ rearrangements, if available.

Common Sequences: Multiple Samples			
Common Sequences			
Clone Tracker	Export	Show 10 entries	
Amino Acid	Total	TCRB_Control1	TCRB_Control2
CASSPRLAGDNEQFF	20,976	144	20832
CASSDGSSTDTQYF	13,410	1812	11598
CASSLGLSSYNEQFF	9,948	78	9870
CASSLWGNQOFF	8,736	114	8622
CASRESNQPHF	8,124	174	7950
CASSLAGTPYEYF	4,878	138	4740
CASSLGENTEAF	3,756	3000	756
CASSKGQEGTEAF	3,582	54	3528
CASSLAATNEKLF	3,432	114	3318
CASSTGTTDTQYF	3,354	24	3330

Showing 1 to 10 of 1,811 entries

First Previous 1 2 3 4 5 Next Last

Click the orange “Initiate Analysis” button at the bottom of the page. To export the results, click the dark blue “Export” button to the upper left of the table, which will then download the file in a .zip format. See [Section 3.6, “Viewing exported data.”](#)

3.4.12 SAMPLE SUMMARY

Sample Summary is available for two or more samples of any locus. It returns a table with a per-sample summary of the types and quantities of sequences observed, as well as some overall sample statistics. Select your samples in the “Choose Samples” box and click the “Sample Summary” icon in the “Choose Analysis” box. You have three options: Show Max Frequency, which includes the maximum frequency for each sample, Gene Rearrangements (number of rearranged genomes present), and Filter DJ Sequences, which is by default checked to filter out DJ sequences, leaving only VDJ rearrangements. Click the orange “Initiate Analysis” button toward the bottom of the page.

Sample Summary: Multiple Samples

Sample Summary

Export

Filters	Sequences	% of Total Sequences	Unique Sequences	% of Total Unique Sequences
Productive	16,315,773	85.625%	214,942	84.966%
Out of frame	2,502,185	13.132%	33,936	13.415%
Has stop	236,870	1.243%	4,097	1.62%
Total	19,054,828		252,975	

Individual Sample Summary

Export Show 10 entries

Sample Name	Total	Unique	Productive Total	Productive Uniques	Out of Frame Total	Out of Frame Uniques	Has Stop Total	Has Stop Uniques	Entropy	Clonality	Max Frequency	Gene Rearr.
TCRB_Control1	6,601,122	79,496	5,769,622	68,872	753,684	9,808	77,616	816	15.034514	0.06453084	1.082%	0
IGH_Memory_Control2	1,591,482	39,671	1,395,794	33,661	166,007	5,045	29,691	965	14.443698	0.039570477	0.347%	47,65
TCRB_Control2	9,647,898	95,622	8,078,094	79,690	1,459,722	14,467	110,082	1,465	14.29152	0.12225631	1.504%	0
IGH_Memory_Control1	1,214,326	38,186	1,072,073	32,719	122,772	4,616	19,481	851	14.412711	0.039014272	0.359%	45,03

Showing 1 to 4 of 4 entries

FirstPrevious1NextLast

You will see two tables. The top table is a summary of all of the combined samples. The bottom table is a summary of each individual sample. The all-samples summary shows the numbers and percentages of total and unique sequences for productive and non-productive clones. The individual sample summary shows more details about each sample including numbers of total and unique sequences that are both productive and non-productive, as well as entropy, clonality, maximum frequency, and gene rearrangements. To see all columns in the bottom table, you may need to place your cursor over the table and scroll to the right. You can export the tables via the dark blue “Export” button to the upper left of each table. The tables will export as a .tsv file.

3.4.13 SAMPLE EXPORT

Sample Export is available for any number of samples. Select the samples you want to export in the “Choose Samples” box and click the “Sample Export” icon under “Choose Analysis.” You may need to scroll down to view this option. For IGH samples, you can select whether to include DJ

sequences (by default these are filtered out and you will only view V(D)J sequences).

Click the orange “Initiate Analysis” button at the bottom of the page. The file will be downloaded as a zip file. It can be expanded into a folder that contains one .tsv file per sample. These tab-separated value files (.tsv files) can be opened using a program such as Microsoft Excel. You must set Excel to enable all files to be opened, not just “readable” files.

The column headers in the exported data are defined in the table below:

Column title	Definition
nucleotide	Full length nucleotide sequence
aminoAcid	CDR3 amino acid sequence
count	The count of sequence reads for the given nucleotide sequence
frequencyCount	The percentage of a particular nucleotide sequence in the total sample
cdr3Length	Total length of the CDR3 region
vMaxResolved	The highest degree of specificity obtained when identifying the V gene
vFamilyName	Name of the V family (if identified)
vGeneName	Name of the V gene if identified, otherwise 'unresolved'
vGeneAllele	Number of the V gene allele if identified, otherwise '1'
vFamilyTies	Name of the potential V families for a sequence if V family identity is unresolved
vGeneNameTies	Name of the potential V genes for a sequence if V gene identity is unresolved
vGeneAlleleTies	Name of the potential V gene alleles for a sequence if V allele identity is unresolved
dMaxResolved	The highest degree of specificity obtained when identifying the D gene
dFamilyName	Name of the D family (if identified)
dGeneName	Name of the D gene if identified, otherwise 'unresolved'
dGeneAllele	Number of the D gene allele if identified, otherwise '1' or 'unresolved' if D gene is unresolved
dFamilyTies	Name of the potential D families for a sequence if D family identity is unresolved
dGeneNameTies	Name of the potential D genes for a sequence if D gene identity is unresolved
dGeneAlleleTies	Name of the potential D gene alleles for a sequence if D allele identity is unresolved
jMaxResolved	The highest degree of specificity obtained when identifying the J gene
jFamilyName	Name of the J family (if identified)
jGeneName	Name of the J gene if identified, otherwise 'unresolved'
jGeneAllele	Number of the J gene allele if identified, otherwise '1'
jFamilyTies	Name of the potential J families for a sequence if J family identity is unresolved
jGeneNameTies	Name of the potential J genes for a sequence if J gene identity is unresolved

jGeneAlleleTies	Name of the potential J gene alleles for a sequence if J allele identity is unresolved
vDeletion	The number of deletions from the 3' end of the V segment
n1Insertion	The number of insertions in the N1 region
d5Deletion	The number of deletions from the 5' end of the D segment
d3Deletion	The number of deletions from the 3' end of the D segment
n2Insertion	The number of insertions in the N2 region
jDeletion	The number of deletions from the 5' end of the J segment
vIndex	Distance from the start of the sequence (designated 0) to the conserved cysteine residue in the V motif
n1Index	Distance from 0 to the start of the N1 region
dIndex	Distance from 0 to the start of the D region
n2Index	Distance from 0 to the start of the N2 region
jIndex	Distance from 0 to the start of the J region
estimated NumberGenomes	Estimated number of T/B cell genomes present in the sample
sequenceStatus	Whether the nucleotide sequence generates a functional amino acid sequence, or a sequence with a stop codon or out-of-frame sequence
cloneResolved	The gene segments used to construct the CDR3 (VDJ, VJ, DJ)
vOrphon	Not implemented at this time
dOrphon	Not implemented at this time
jOrphon	Not implemented at this time
vFunction	Not implemented at this time
dFunction	Not implemented at this time
jFunction	Not implemented at this time

3.4.14 EXPLORE EDIT DISTANCE

Explore Edit Distance is available for any single sample and visually represents the relationship between clones. To run this analysis, first select your sample in the “Choose Samples” box and then click the “Explore Edit Distance” icon in the “Choose Analysis” box. There are no configuration options for this analysis type, so simply click the orange “Initiate Analysis” button at the bottom of the page. The pull-down menus will give you the option to choose among particular V and J gene and CDR3 lengths and will show the number of unique clones fitting those criteria. These clones are represented by multicolored balls. The pull-down menu on the right will bin the clones based on the number of substitutions between the clones. This changes the representation by drawing a line between the multicolored balls.

3.4.15 SAMPLE OVERLAP

Sample Overlap is available for at least two to as many as 25 samples of any locus. It produces both an overlap metric as well as a Pearson's correlation (r^2) value for each possible pair-wise percent sharing between all pairs of samples. This is computed by averaging across the two ratios of shared reads over total reads for each sample. The correlation of frequencies for all shared clones is given by the r^2 .

	IGH_Memory_Control1	IGH_Memory_Control2	IGH_Naive_Control1	IGH_Naive_Control2
IGH_Memory_Control1		$r^2: 0.710$ $\alpha: 0.215$	$r^2: 0.000$ $\alpha: 0.004$	$r^2: 0.006$ $\alpha: 0.005$
IGH_Memory_Control2	$r^2: 0.710$ $\alpha: 0.215$		$r^2: 0.014$ $\alpha: 0.005$	$r^2: 0.011$ $\alpha: 0.006$
IGH_Naive_Control1	$r^2: 0.000$ $\alpha: 0.004$	$r^2: 0.014$ $\alpha: 0.005$		$r^2: 0.254$ $\alpha: 0.007$
IGH_Naive_Control2	$r^2: 0.006$ $\alpha: 0.005$	$r^2: 0.011$ $\alpha: 0.006$	$r^2: 0.254$ $\alpha: 0.007$	

You can order the overlap map by Sample Name, by Overlap, or by r^2 .

To run this analysis, first select your samples in the “Choose Samples” box and then click the “Sample Overlap” icon in the “Choose Analysis” box. The parameters available to select are as follows:

Compare Using: Nucleotide, Amino Acid, V, J, and Amino Acid Only.

Base Pairs to Match: Entire length vs. an input amount (if using Nucleotide to compare).

Match Specificity: V Gene or V Family (if using Amino Acid, V, J to compare).

Status Code: Productive, Out Of Frame, Has Stop (can choose multiple options).

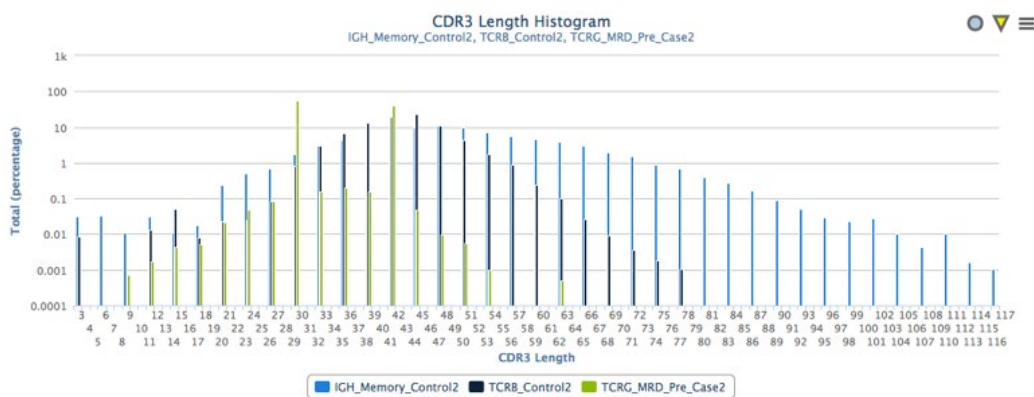
Include Alleles in Query: This makes the match more stringent. Both the gene and alleles must match.

Filter DJ Sequences: By default the DJ rearrangements are filtered out. Uncheck this box to include them.

Click the orange “Initiate Analysis” button at the bottom of the page. Initially you will see a map ordered by sample name. Overlap and r^2 will be listed in each square. Use the pull-down menu at the upper left to re-order the graph by overlap or by r^2 .

3.4.16 CDR3 LENGTH

CDR3 Length analysis can be used for any number of samples of any locus. It returns a histogram of CDR3 length vs. counts or sample percentage. If you would like a single-sample histogram of CDR3 length for a specific human TCRB family, use the “SpectraSEQ” analysis option instead (See [Section 3.4.4, “SpectraSEQ”](#)).



To run this analysis, first select your samples in the “Choose Samples” box, and then select the “CDR3 Length” icon from the “Choose Analysis” box. To find this icon, scroll to the bottom of the “Choose Analysis” options box. You will have several options to select from in the “Choose Parameters” box.

Select whether the y-axis information is expressed in total or unique counts. If you select “Total” counts, the histogram will be drawn based on the total number of counts observed per sample.

If you select “Unique” counts, the histogram will be drawn based on the number of unique sequences observed per sample. By default, the y-axis is displayed in percentages of total counts. When displaying as percentages, select whether it is percentage of total counts or just the percentage of the filtered sequences. You can select whether or not to use a logarithmic scale for the y-axis.

Next, you can select whether to use only “Productive” sequences (this is the default), or also include “Out Of Frame” and/or “Has Stop” sequences. Lastly, select whether to filter out DJ rearrangements (this is the default), leaving only VDJ rearrangements. To include them, uncheck the filter box.

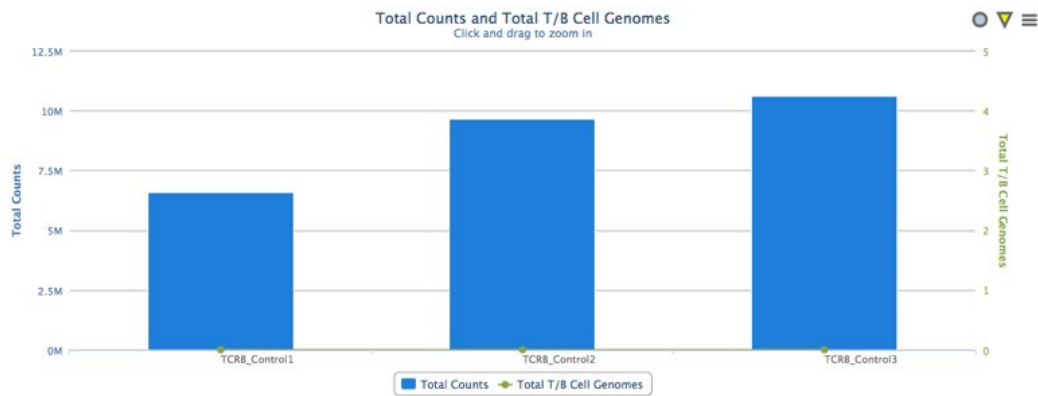
After making your selections, click the orange “Initiate Analysis” button at the bottom of the page to generate the histogram.

By positioning your cursor over any of the histogram bars, a pop-up window appears detailing the CDR3 length, sample name, counts, and a breakdown of how many of those counts are in-frame, out-of-frame, or have a stop.

3.4.17 QUALITY CONTROL

Quality Control analysis is available for any number of samples of any locus. You will see two overlaid line/bar graphs for each sample. At the top is a bar graph of total counts with a line graph of Gene Rearrangements, and at the bottom is a bar graph of unique counts, one of unique counts with a line graph of clonality. The default blue color and left y-axis are for the bar graph data; the default green color and right y-axis are for the line graph data. By hovering over each bar, a pop-up window appears with the sample name and the values for Total Counts, Gene Rearrangements, Unique Counts, and Clonality, as appropriate.

To run this analysis, first select your sample(s) and then select the “Quality Control” icon from the “Choose Analysis” box. You have two options in the “Choose Parameters” box: whether to use a Logarithmic Scale on the y-axis and whether to filter out the DJ rearrangements (this is the default), leaving just the VDJ rearrangements. To include them, uncheck the box. The click the orange “Initiate Analysis” button at the bottom of the page.



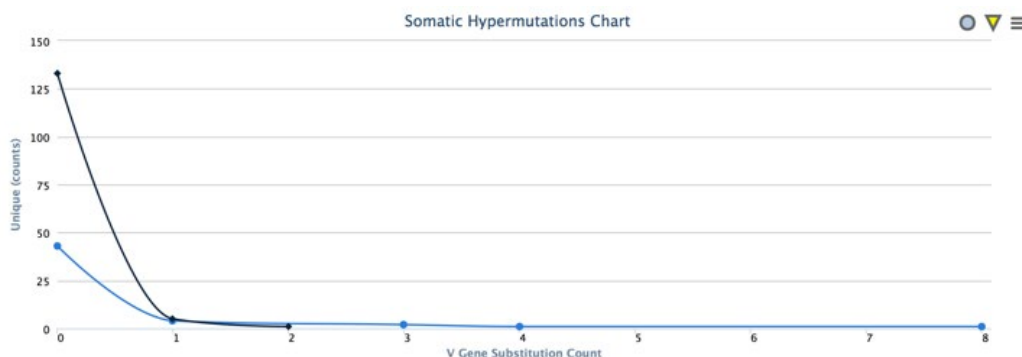


3.4.18 SOMATIC HYPERMUTATION

The Somatic Hypermutation analysis feature provides a count of point mutations in the V gene for IGH and IGKL samples. This feature is available for samples run after July 2014.

To perform this analysis, go to the Analysis section, select your samples, and scroll down in the “Choose Analysis” window until you see the “Somatic Hypermutation” icon. Select the icon and then choose parameters.

You can choose to view the y-axis as unique or total counts. (Unique will give you a count of the number of unique sequences with a given number of point mutations, and Total will sum up the total number of observed reads for all of the sequences.) You can also choose display the y axis as percent or count or in logarithmic scale. The “Status Code” option allows you to include Productive, Out Of Frame, and/or Has Stop sequences as you wish, and to filter DJ sequences. In this example productive uniques have been selected. After making your selections click “Initiate Analysis”.



You will get a chart with the number of sequences (unique or total) on they y axis, and the V gene substitution count on the x axis. Each colored line represents a different sample. If you hover over each of the points in the chart with your mouse, a popup will appear detailing the sample name, number of substitutions, and count. The example chart shows the number of substitutions in productive, unique sequences.

3.4.19 QC REPORT

The QC report contains metrics that allow you to assess the quality of your sequencing data, including sequencing coverage and number of sequencing reads obtained for each sample. The QC report can be used to validate the success of each sample, and to confirm that all samples in a project have been processed the same way.

QC Report: Multiple Samples

QC Report

Export

Show 10 of 6 entries

Sample Name	Kit ID	Run ID	Well Location	Date Plated	Total Reads	Gene Rearrangements	Coverage	% On target	QC Status	Assay Version	Pipeline Version
10080_CD4_Tcr-Kit	NA	NA	A1	10/21/2014	215,582	215,582	20.6	0	Pass	2.0	NA
10080_CD4_Tcr-Kit	NA	NA	A2	10/21/2014	188,659	188,659	20.5	0	Pass	2.0	NA
10080_CD4_Tcr-Kit	NA	NA	A8	10/21/2014	92,075	92,075	20.9	0	Pass	2.0	NA
10080_CD4_Tcr-Kit	NA	NA	A10	10/21/2014	52,817	52,817	20.6	0	Pass	2.0	NA
10080_CD4_Tcr-Kit	NA	NA	A12	10/21/2014	88,755	88,755	19.9	0	Pass	2.0	NA
10080_CD4_Tcr-Kit	NA	NA	A11	10/21/2014	83,419	83,419	22.0	0	Pass	2.0	NA

Showing 1 to 6 of 6 entries

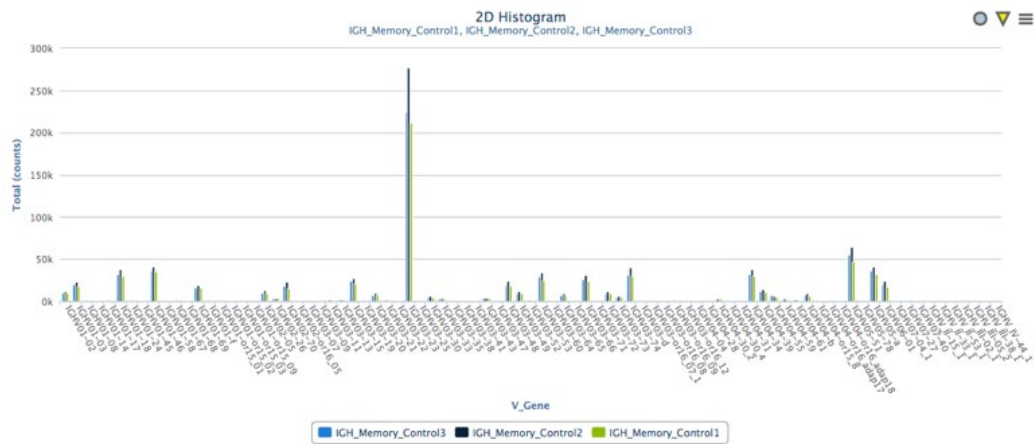
3.5 MODIFYING AND EXPORTING ANALYSIS FIGURES

At the upper right corner of a figure resulting from an analysis, you will see three symbols: a circular icon, meaning “Customize Your Chart”, a downward-facing arrow, “”, meaning “Export Chart Data”, and a document icon, meaning “Export Chart Image”.

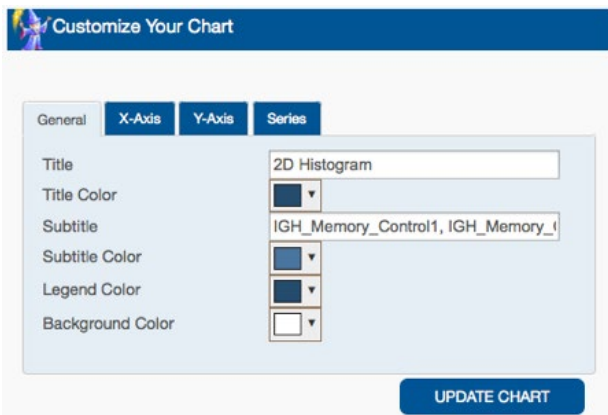


3.5.1 CUSTOMIZE YOUR CHART

Demonstrated for 2D histogram “Gene Frequency” analysis:



When you click the circular icon, a pop-up window appears with four tabs with options for customizing your chart. Once you make your selections, click the “UPDATE CHART” button.



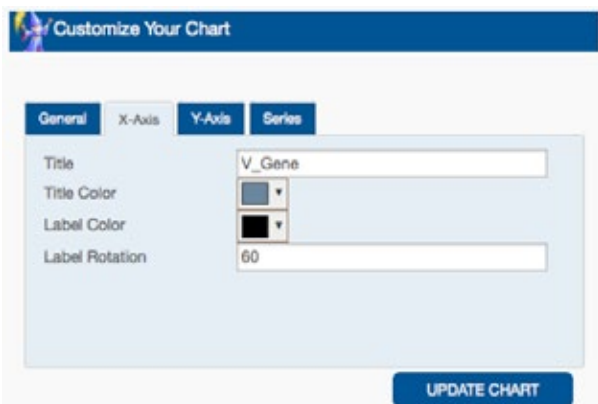
The screenshot shows the 'Customize Your Chart' dialog box with the 'General' tab selected. The dialog has four tabs: General, X-Axis, Y-Axis, and Series. Under the General tab, there are six settings: Title (text box with '2D Histogram'), Title Color (color picker with a dark blue square), Subtitle (text box with 'IGH_Memory_Control1, IGH_Memory_I'), Subtitle Color (color picker with a light blue square), Legend Color (color picker with a dark blue square), and Background Color (color picker with a white square). An 'UPDATE CHART' button is located at the bottom right.

Under the General tab, use the fill-in boxes to edit the title and subtitle. Change the color of the title, subtitle, legend text, and figure background by using the pull-down menus, clicking on a color family using the vertical bar, then clicking a specific color within the square (see image below), and finally clicking “Choose.”



The screenshot shows a color picker dialog box. It features a large square color field with a gradient from light blue to dark blue. To the right of the field is a vertical color bar with a rainbow spectrum. Below the color field is a text box containing the hex code '#77bebe'. At the bottom are two buttons: 'cancel' and 'choose'.

The X-Axis tab allows you to change the x-axis title, colors of title and label, and the label rotation. Label rotation is in degrees and you may enter any number you wish. A rotation of 90 degrees will display the labels vertically. A rotation of 0 degrees will display the labels horizontally.



The screenshot shows the 'Customize Your Chart' dialog box with the 'X-Axis' tab selected. The dialog has four tabs: General, X-Axis, Y-Axis, and Series. Under the X-Axis tab, there are four settings: Title (text box with 'V_Gene'), Title Color (color picker with a light blue square), Label Color (color picker with a black square), and Label Rotation (text box with '60'). An 'UPDATE CHART' button is located at the bottom right.

The Y-Axis tab allows you to change the y-axis title, colors of title and label, and label rotation. As with the x-axis, a rotation of 0 degrees displays the labels horizontally and a rotation of 90 degrees displays the labels vertically. You may also enter a Min and Max value for the y-axis. In some cases, the values may default to the next highest nearest major axis interval. For example, if the y-axis is marked in intervals of 50,000, a Max Value of 240,000 or 210,000 may both default to 250,000.

The screenshot shows the 'Customize Your Chart' dialog box with the 'Y-Axis' tab selected. The 'General' tab is also visible. The 'Y-Axis' tab contains the following fields:

- Title: Total (counts)
- Title Color: (Color selection dropdown)
- Label Color: (Color selection dropdown)
- Label Rotation: 0
- Min Value: 0
- Max Value: (Empty field)

An 'UPDATE CHART' button is located at the bottom right of the dialog box.

In the Series tab, you can change the colors of the individual data series shown in your figure. In this case, the histogram bars can change color.

The screenshot shows the 'Customize Your Chart' dialog box with the 'Series' tab selected. The 'General' tab is also visible. The 'Series' tab contains the following series names and color selection dropdowns:

- IGH_Memory_Control3: (Blue color selection dropdown)
- IGH_Memory_Control2: (Dark blue color selection dropdown)
- IGH_Memory_Control1: (Green color selection dropdown)

An 'UPDATE CHART' button is located at the bottom right of the dialog box.

In the case of figures other than histograms, your choices will vary slightly. For example, in the case of a pie chart, you will see only two tabs: General and Series. If you are unable to see all of the included series in the "Series" tab, position your cursor over the series names and begin to scroll down. A scroll bar will appear and you will be able to scroll as needed. In the case of scatter plots, you will be able to select the symbol shape and size.

3.5.2 DOWNLOAD FIGURE DATA

Click the downward-facing arrow to download a tab-separated value (.tsv) file with the underlying data from the figure. To open the .tsv file, use a spreadsheet program such as Microsoft Excel and make sure that it is set to open all files (not just all readable files). Excel will not automatically recognize the .tsv extension but with the "open all files" option set, it will open and process the file.

3.5.3 EXPORT A FIGURE

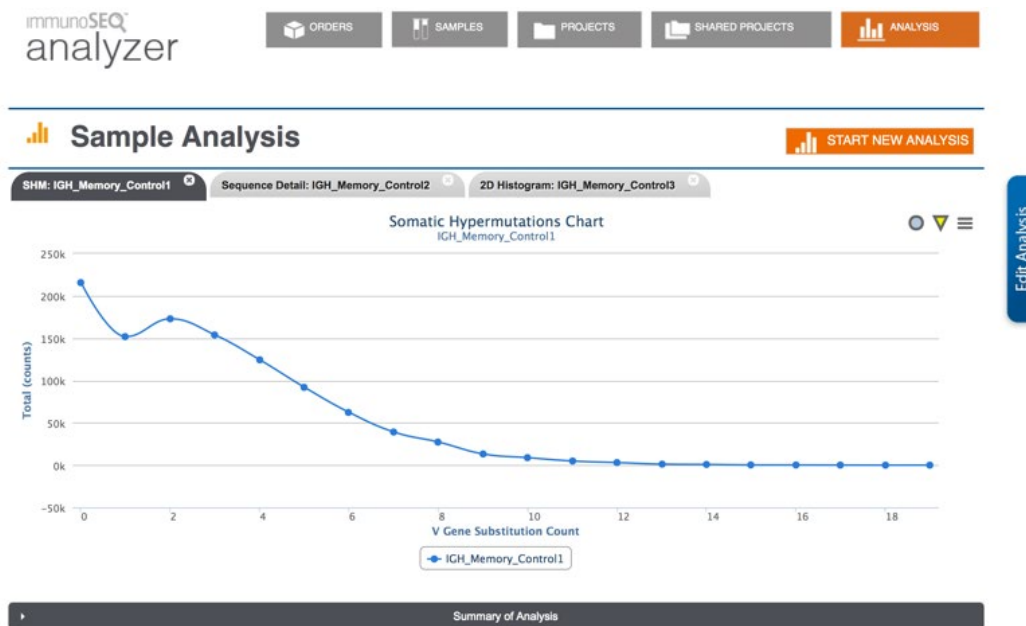
To export a high-quality image of the figure, click on the document icon. A pop-up window will appear and you can click on either “Export to PDF,” “Export to PNG,” or “Export to JPEG.” After you click the file choice, the figure will automatically download.

3.6 VIEWING EXPORTED DATA

To open a .tsv file, use a spreadsheet program such as Excel and make sure that it is set to open all files (not just all readable files). Microsoft Excel will not automatically recognize the .tsv extension but with the open all files option set, it can open and process the file.

3.7 EDITING AN ANALYSIS

If you would like to do a new analysis, but still see be able to access the results of a previous analysis, click the “Start New Analysis” button. This will make the results of your new analysis appear in a new tab, and you will be able to use the tabs above the analyses to switch between results. Use the “x” in the upper right corner of the tab to close the tab. If you click the main Analysis button to do a new analysis, it will clear all of your previous analyses.

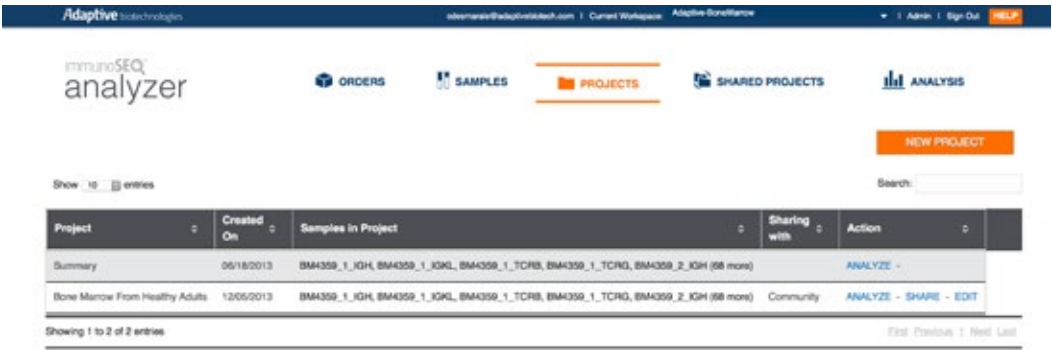


To remind yourself of the parameters you selected for any particular analysis, click the “Summary of Analysis” bar at the bottom of the analysis results. If you would like to change the analysis parameters for a certain analysis, click the “Edit Analysis” tab at the right of the results. Then if you click “Update Analysis”, the results in that tab will be updated. If you instead click “Initiate Analysis”, a new tab will be created with the new results.

You can also edit a figure by clicking on any series in the legend. The series will be removed from the graph or figure and the name will be greyed-out in the legend, but still present. You can re-insert it into the figure simply by clicking on the greyed out series name.

4. Manage My Projects

The Projects page allows the user to associate samples with individual projects. A project is a customer defined set of samples. Samples may come from the customer's workspace summary project or the publicly shared community samples. This allows for the selection of a subset of samples for analysis, which can be highly useful for users who have a large number of samples. A single sample can be associated with multiple projects.

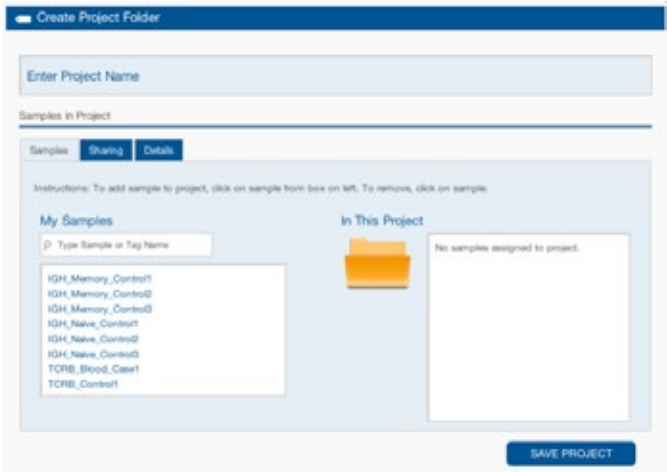


4.1 SUMMARY PROJECT

Summary is the default project for every workspace. It contains all of the samples that belong to the workspace. It does not contain samples belonging to an open-access project that you are following.

4.2 CREATING A NEW PROJECT

To easily work with a subset of your data, you can create projects that include only selected samples. To create a new project, click the orange “New Project Folder” button towards the upper right corner of your screen. This will bring up the “Create Project” dialog box.



Fill in your new Project name in the light blue “Enter Project Name” box toward the top of the page.

To add a sample to the project, click on the sample name in the box on the left. This will move the sample to the project box on the right. To remove a sample, click on the sample name in the box on the right. If you place a sample in a project, it will still be available in the “Summary” section and you can place it in other projects.

Save the new project by clicking the orange “Save Project” button at the lower right.

4.3 SHARING A PROJECT

You can share a project with specific colleagues or with the community at large via the “Sharing” tab in the “Create Project Folder” dialog box. To share with a specific colleague, enter his or her email address and click the Add button. When you click “Save Project,” you will be asked to confirm the project you want to share. See [Section 6, “Shared Open Access Projects”](#).

4.4 PROJECT DETAILS

You can enter project details using the “Details” tab in the “Create Project Folder” dialog box. Details that can be added include institution, investigator, contact information, URL, research area, and description.

4.5 EDITING A PROJECT

To edit an existing project, click on the “Project” tab and scroll all the way to the right to find the “Action column” button on the dark blue column heading. (Depending on your screen size, the “Action column” button may not appear on your screen until you scroll to the right.) Click the Edit link in the Action column. This opens the Edit Project dialog. It brings up the same dialog box as when you created the project. In “Samples” you can change the samples; in “Sharing” you can add or remove colleagues (to remove colleagues, simply hover over their email address and click the “X” that appears to the right). Your changes are automatically saved when you close the dialog box by clicking the clicking the Save.

5. Manage My Samples

The Samples button brings you to a table where you can view details of each individual sample in your workspace. The columns included are: Samples, Projects, Sharing with, Tags, and Action. You can search for a particular sample using the search box in the upper right. Use the pull-down menu on the upper left to change the number of entries you can view. Columns can be sorted by clicking on the column headings.

The screenshot shows the 'Samples' tab of the immunoSEQ Analyzer. The table has columns for Samples, Projects, Sharing with, Tags, and Action. Each row represents a sample with details on its name, species, locus, sequencing depth, project affiliation, sharing status, and available actions (Analyze, Share, Tag). The 'Tags' column contains a series of orange buttons representing different data points or markers.

Samples	Projects	Sharing with	Tags	Action
BM4359_1_IGH Human, IGH, Deep	Bone Marrow From Healthy Adults, Summary	Community	IGH-IGH Early Adulthood (18-30yrs) IGH-IGH IGH-IGH IGH-IGH IGH-IGH IGH-IGH IGH-IGH	ANALYZE - SHARE - TAG
BM4359_1_IGK Human, IGK, Deep	Bone Marrow From Healthy Adults, Summary	Community	IGH-IGH Early Adulthood (18-30yrs) IGH-IGH IGH-IGH IGH-IGH IGH-IGH IGH-IGH IGH-IGH	ANALYZE - SHARE - TAG
BM4359_1_TCRB Human, TCRB, Deep	Bone Marrow From Healthy Adults, Summary	Community	IGH-IGH Early Adulthood (18-30yrs) IGH-IGH IGH-IGH IGH-IGH IGH-IGH IGH-IGH IGH-IGH	ANALYZE - SHARE - TAG
BM4359_1_TCRG Human, TCRG, Deep	Bone Marrow From Healthy Adults, Summary	Community	IGH-IGH Early Adulthood (18-30yrs) IGH-IGH IGH-IGH IGH-IGH IGH-IGH IGH-IGH IGH-IGH	ANALYZE - SHARE - TAG

5.1 BASIC SAMPLE DETAILS

In the first column, you will see the sample name. In light color below, you will see the species, locus, and sequencing depth associated with that sample. If the sample is still being processed and the data are not yet available, you will also see “Sequence Results Pending” below the sample name. For recently run samples, when the data is available you will see “Released” as well as the date the data was made available.

5.2 PROJECT AFFILIATION

The “Projects” column shows the projects that the samples have been grouped into. All samples fall under the “Summary” project by default. If placed in additional project(s), the name(s) of those project(s) will be listed next to “Summary.” See [Section 4, “Manage My Projects”](#) for more details. If, for any sample, you click on the Analyze link for a project in the Actions column, you will be redirected to the Analysis page, with that project already selected as a filter. To go back to another tab or cancel this action, click on the “Cancel” button.

5.3 SHARING A SAMPLE

If you have already shared a sample, the “Sharing with column” will indicate either “colleagues” or “community”, depending on whom the sample has been shared with.

Click the Share link to share with colleagues or the community. This box allows you to select one of the projects that the sample belongs to, or to create a new project folder. If you select an existing project, you will be redirected to the “Sharing” tab of the “Manage Folder” dialog box for that folder so that you can edit your sharing choices (See [Section 4.3, “Sharing a Project”](#)). If you select “Create Project Folder,” you will be redirected to the “Sharing” tab of the “Create Project Folder” dialog box (See [Section 4.2, “Creating a New Project”](#)). The sample that you have selected will be the only sample in the new project, and you will need to name the project and fill in the sharing information.

bottom of the “Edit Tags” box. Our team of scientists will need to approve and add this tag before it shows up as an option in the immunoSEQ tag library.

Once you have located an appropriate tag from one of the eight categories, click on the tag to send it to the “Applied Tags” box. If you would like to remove a tag, click on the tag in the “Applied Tags” box. You may apply multiple tags to each sample. Your selections will be saved when you click the “Save Tags” button a the bottom of the box.

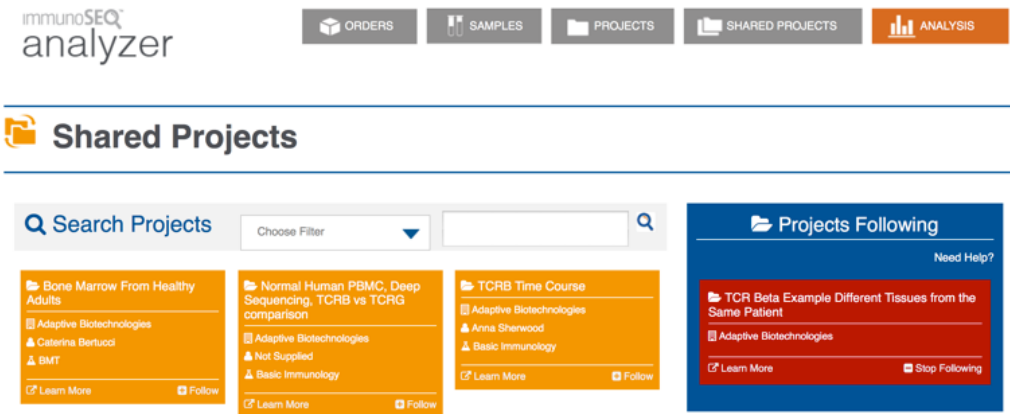
5.6 EXPORTING SAMPLE INFORMATION

If you click on the “Export” button to the upper left of the table, you will download a tab-separated values (.tsv) file with the contents of the table. The file contains columns including: Sample, Species, Locus, Product, Release Date, Upload State, Project and Tag information.

6. Shared Open Access Projects

To view projects shared with the community, click on the “Shared Projects” button in the top set of five main buttons.

Shared projects are a way for the user to collaborate with colleagues and to utilize data from Adaptive Biotechnologies’ library of community projects. Projects on the left side are projects that have been shared with the immunoSEQ community and are available to follow. Projects on the right are projects that you are currently following. These can include projects that a colleague has invited you to share or immunoSEQ community projects that you have chosen to follow. All currently followed projects will show up under the project filter on the analysis page where you can use one of immunoSEQ’s analysis tools to visualize that project’s sample data.

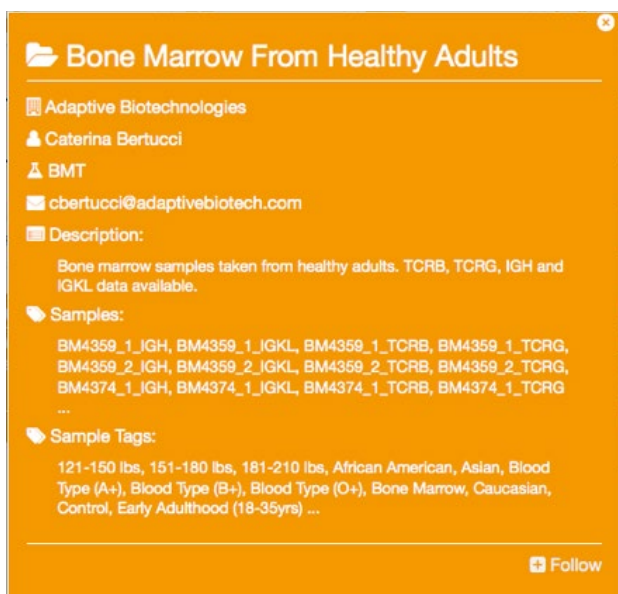


6.1 SEARCHING FOR A PROJECT

To search for a project, first select a category. To do this, click on the inverted blue triangle to reveal the pull-down menu “Choose Filter”. Next, type your search term in the box to the right and click the small magnifying glass icon under “Search Projects.”

6.2 FOLLOWING A PROJECT

Initially you will see the available projects under the “Search Projects” label. If you want to know more details, click the “Learn More” icon at the bottom of the left orange box and a pop-up window will list detailed project information including institution, PI, research category, contact information, description, samples, and sample tags.



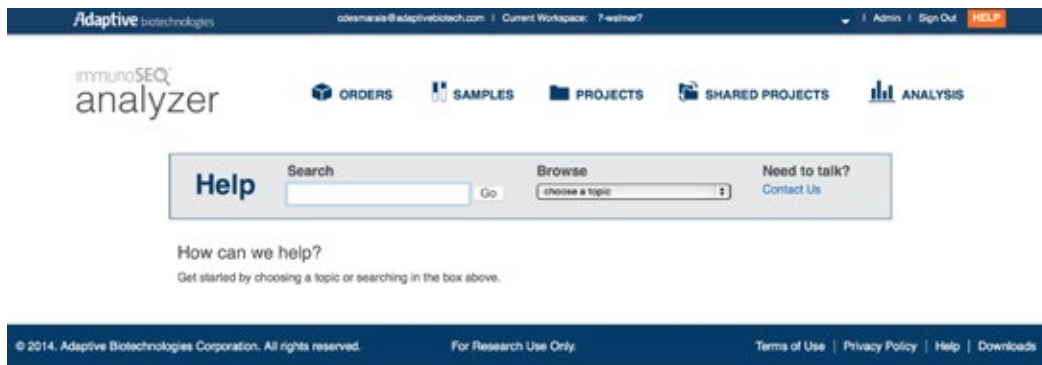
Close the pop-up window by clicking the “x” in its upper right corner. To be able to analyze a project’s samples on your analysis page, click “follow” either on the initial page or in the lower right-hand corner of the pop-up window. The project summary will be moved to the right side of the page in the “Projects Following” box. To stop following a project, click “Stop Following”, located in the lower right-hand corner of the orange box.

6.3 SHARE YOUR PROJECT

If you wish to share a project with the community, first click on the “Projects” link in immunoSEQ. Scroll to the rightmost column and click on the “Manage Folder” button. Use the “Sharing” tab in the pop-up window and click on the checkbox next to “Share with the ImmunoSEQ community”. At the bottom right click “Save Project”. Your project will be submitted to Adaptive Biotechnologies for consideration.

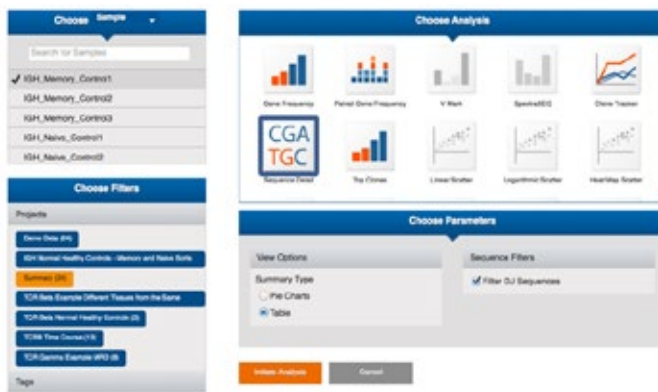
7. Request a Feature

To request a new immunoSEQ feature, click on the “Help” link in the blue bar at the bottom of each page. Click the Contact Us link on the main Help page and let us know what features you’d like to see in upcoming versions of the Analyzer. Once you’ve filled in the details box, hit Submit and your request will be submitted.

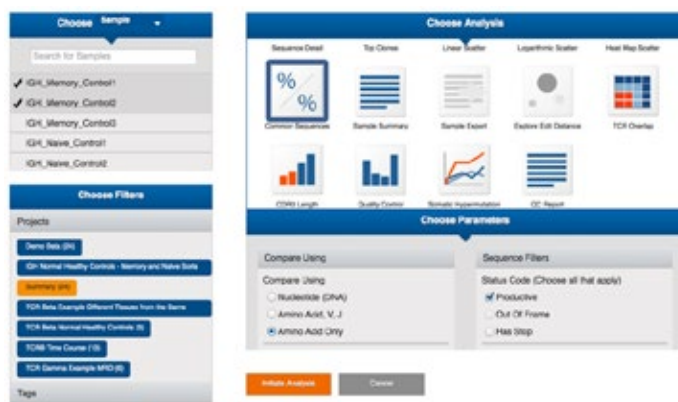


8. Tutorial

- Log in to your account or access the demo site.
- Click on the “Analysis” button.
- Select one sample from the “Choose Samples” panel to use as an example. Look at the “Choose Analysis” panel and note what options are available for a single-sample analysis.
- Select “Sequence Detail” analysis. Click the orange “Initiate Analysis” button at the bottom of the page.



- This Sequence Detail table displays the immunoSEQ assay data on which much of the rest of the analyses are based. Understanding the fields reported here will help in understanding the rest of the analyses. Read through the “Sequence Detail” explanation (See [Section 3.4.6, “Sequence Detail”](#)) while looking at this table.
- Go to “Start New Analysis,” select an example sample again, and select another analysis to try. Then go to the “Edit Analysis” tab on the right side of the page and select yet another analysis. Click through all of the available single-sample analyses to familiarize yourself with the different options that are available.
- Select “Start New Analysis” and choose two example samples. Look at the “Choose Analysis” panel and note what options are available for two-sample analysis. (The only exclusively two-sample analyses are the scatter plots.) Click on the “Common Sequences” analysis to see the data on which the scatter plots are based. Read through the “Common Sequences” explanation (See [Section 3.4.11, “Common Sequences”](#)) while looking at this table. Then go to “Edit Analysis” on the right side of the page and try the scatter plot analyses.
- Choose “Start New Analysis” and select three example samples. Look at the “Choose Analysis” panel and note what options are available for three (or more) samples. Click again on the “Common Sequences” analysis to see the data on which much of the rest of the analyses are based. Then go to the “Edit Analysis” tab on the right side of the page and try out the various 3-sample analyses.



- Lastly, click on the “Samples” and “Projects” buttons to understand each way to view the sample lists.

9. Index

Alleles, 10, 11, 13, 20, 22, 24, 25, 26

Assay Depth
Deep, 10

CDR3, 5, 6, 7, 15, 17, 20, 24, 25, 26, 27

Clonality, 6, 7, 17, 18, 23, 27

Clone, 5, 6, 7, 15, 16, 17, 18, 19, 20, 22, 23, 25

Common Sequences, 5, 22, 39

Community Page, 33, 34, 35, 37, 38

Counts, 6, 7, 11, 13, 14, 17, 20, 21, 22, 26, 27, 28

Customize plots, 5, 9, 29, 36

Deep, 10

DJ rearrangement, 11, 13, 14, 15, 17, 18, 20, 22, 23, 25, 26, 27, 28

Download, 3, 4, 5, 6, 11, 12, 14, 15, 17, 23, 24, 29, 31, 32, 37

Entropy, 7, 17, 23

Export, 3, 4, 5, 6, 11, 12, 14, 15, 17, 23, 24, 29, 31, 32, 37

Family, 6, 7, 10, 11, 15, 24, 26, 30

Filter, 8, 9, 10, 11, 13, 14, 15, 16, 17, 20, 22, 23, 24, 26, 27, 28, 35, 37, 38

Flow Cytometry, 6, 14

Frequency, 5, 6, 7, 9, 10, 11, 12, 13, 15, 16, 18, 23, 29

Gene, 5, 6, 7, 9, 10, 11, 12, 13, 17, 18, 20, 22, 23, 24, 25, 26, 27, 29

Has Stop, 11, 13, 17, 20, 22, 26, 27, 28

IGH, 10, 11, 13, 18, 23, 28

In frame, 27

Intersection, 22

J gene, 6, 7, 10, 11, 18, 20, 22, 24, 25

Locus, 10, 12, 22, 23, 25, 26, 27, 35, 36, 37

Out of frame, 11, 13, 17, 20, 22, 25, 26, 27, 28

Overlap, 5, 7, 21, 25, 26

Pearson's correlation, 7, 20, 25

Plots
Histogram, 4, 9, 10, 11, 12, 13, 14, 15, 19, 26, 27, 29, 31
Scatter, 5, 20, 21, 31, 39

Productive, 11, 13, 17, 20, 22, 23, 26, 27, 28, 29

Projects, 3, 8, 9, 29, 33, 34, 35, 37, 38, 39
Share, 34, 35, 37, 38

Quality Control, 5, 27, 29

Sample Summary, 5, 23

Sequence Status

Has Stop, 11, 13, 17, 20, 22, 26, 27, 28

In frame/productive, 11, 13, 17, 20, 22, 23, 26, 27, 28, 29

Out of frame, 11, 13, 17, 20, 22, 25, 26, 27, 28

Somatic Hypermutation, 5, 18, 28

Spectratyping, 5

T/B cell count, 25

Tags, 9, 10, 16, 19, 34, 36, 37, 38

TCRB, 6, 9, 14, 15, 26

Union, 22

Uniques, 6, 7, 11, 13, 17, 27, 28

Unresolved, 11, 13, 20, 24, 25

V family, 6, 10, 11, 15, 20, 22, 24, 26

V gene, 6, 7, 10, 11, 14, 18, 22, 24, 26, 28, 29

VDJ rearrangement, 11, 13, 14, 15, 17, 20, 22, 23, 25, 27



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