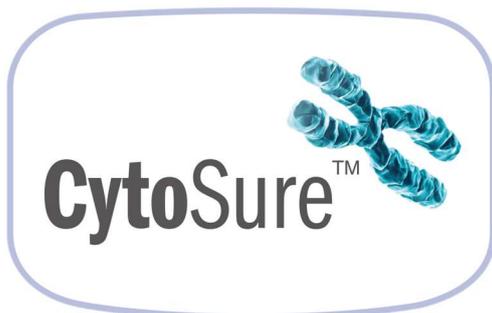




A Sysmex Group Company

Interpret

NGS Analysis Software



Interpret Quick-Start Guide

Oxford Gene Technology

Founded by Professor Sir Ed Southern, Oxford Gene Technology (OGT) supplies world-class genetics research solutions to leading clinical and academic research institutions.

- **CytoSure™** — Class-leading products offering the complete array and NGS solution for clinical genetics research.
- **Cytocell®** — High-quality fluorescence *in situ* hybridisation (FISH) probes for the detection of gene rearrangements related to inherited genetic disease and cancer.
- **SureSeq™** — Hybridisation-based NGS panels and library preparation reagents delivering flexibility and unparalleled coverage uniformity for clinical cancer research applications.

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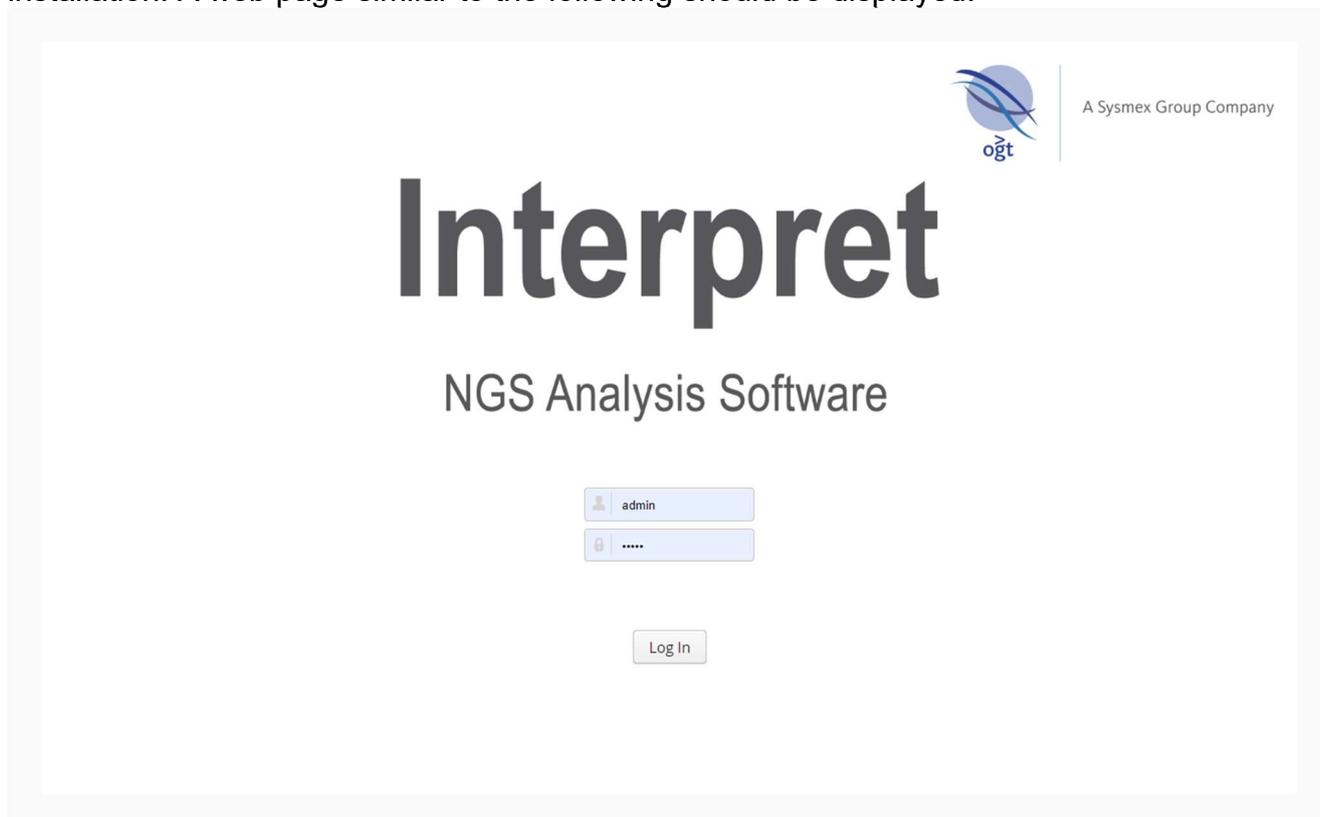
Upload the FASTQ Files

Note: For each sample to be processed by Interpret, the input is a pair of FASTQ files (one for read 1; one for read 2). However, the sequencer may generate multiple FASTQ files for each sample for each lane on which the sample was sequenced. If this is the case, and you do not know how to combine the FASTQs, please contact OGT for support (support@ogt.com).

1. Open Interpret using the **Interpret v3** desktop shortcut. If no such link is provided, open a web browser and navigate to the URL of Interpret, which will take the format:

http://COMPUTERNAME:PORT/sureseq

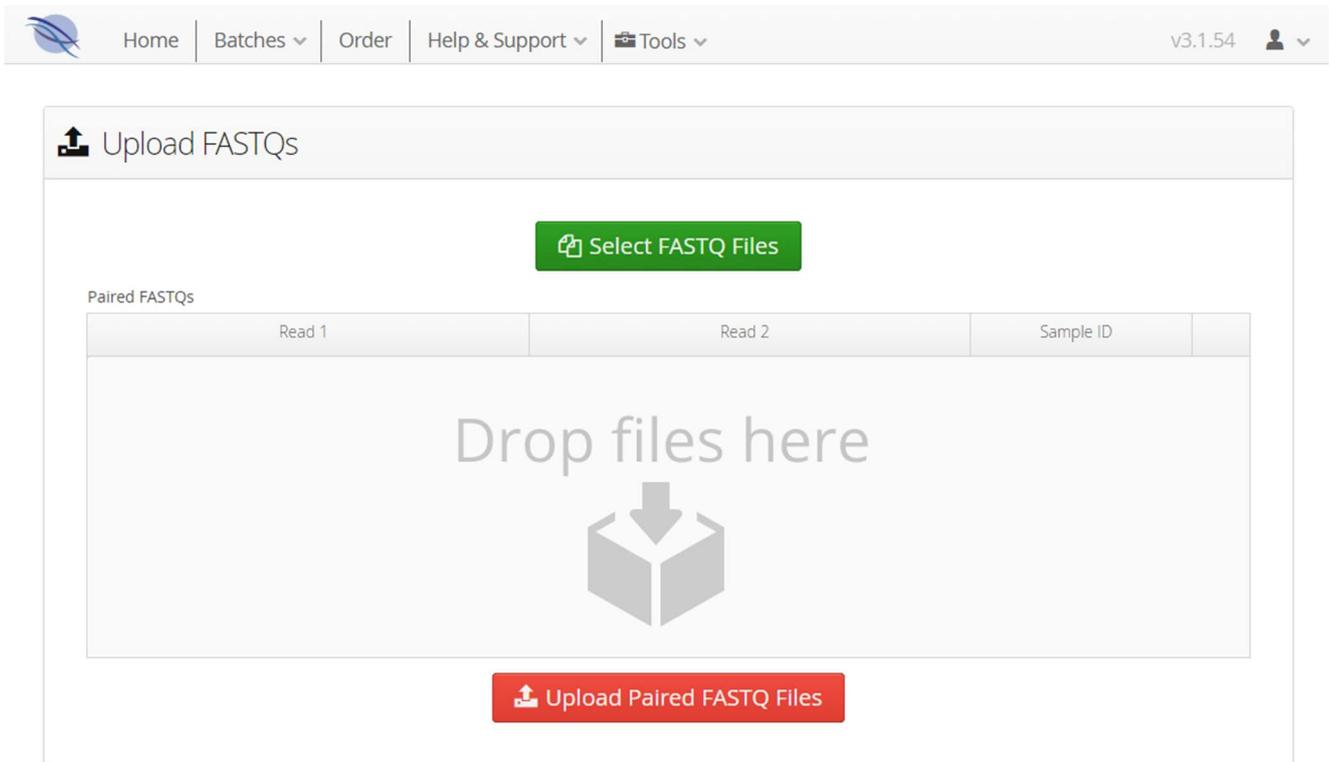
If the software has been installed on the computer in use, but by a different user, the COMPUTERNAME will be "localhost". If it has been installed on another computer on the network, COMPUTERNAME will be the name or IP address of the computer on the network. The default PORT is 80, but may have been changed to something else by the user during installation. A web page similar to the following should be displayed:



2. Login with the user name "admin" and the password provided during installation.



3. Click the **Upload FASTQs** button at the bottom of the screen.



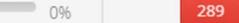
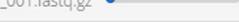
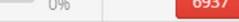
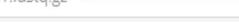
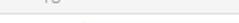
4. Click the **Select FASTQ Files** button, select all the FASTQ Files for any new reference or test samples, and click **Open**. The FASTQ files should be automatically paired and listed in the **Paired FASTQs** table.

Home | Batches ▾ | Order | Help & Support ▾ | Tools ▾ | v3.1.54 | 

Upload FASTQs



Paired FASTQs

Read 1	Read 2	Sample ID	
156_R1.fastq.gz  0%	156_R2.fastq.gz  0%	156	
289_R1.fastq.gz  0%	289_R2.fastq.gz  0%	289	
4315_7_L000_R1.fastq.gz  0%	4315_7_L000_R2.fastq.gz  0%	4315	
6937_6_L001_R1_001.fastq.gz  0%	6937_6_L001_R2_001.fastq.gz  0%	6937	
10384_7_L000_R1.fastq.gz  0%	10384_7_L000_R2.fastq.gz  0%	10384	
10847_7_L000_R1.fastq.gz  0%	10847_7_L000_R2.fastq.gz  0%	10847	
Estimated Time Remaining: ?		Data Uploaded: 0.0 B/18.1 GB (0.00%)	Files Uploaded: 0/18

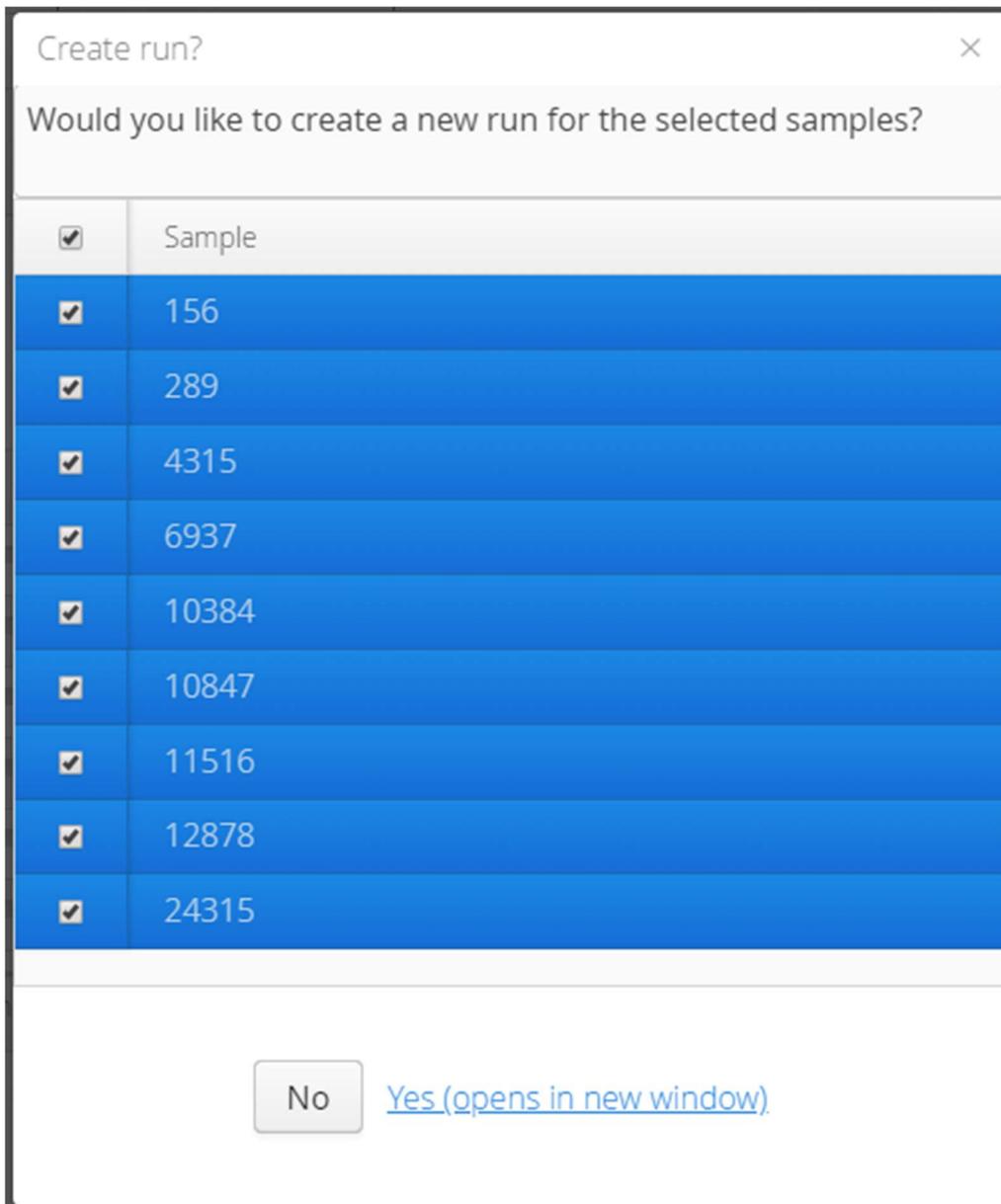


5. Click **Upload Paired FASTQ Files**.

Confirm ✕

All Paired FASTQ files must be assigned to a sample. Would you like to use the default samples IDs (displayed in red in the Sample ID column)? Click Cancel to manually assign samples (by clicking on the defaults).

6. Click **Ok**.



7. Deselect any reference samples for which you do not want to generate CNV/LOH/SNV results, and click **Yes (opens in new window)**. **If using Chrome, separate the new tab into a different window and ensure that the Upload FASTQs tab is selected in the original window. This is essential to ensure that the upload continues in the background.**

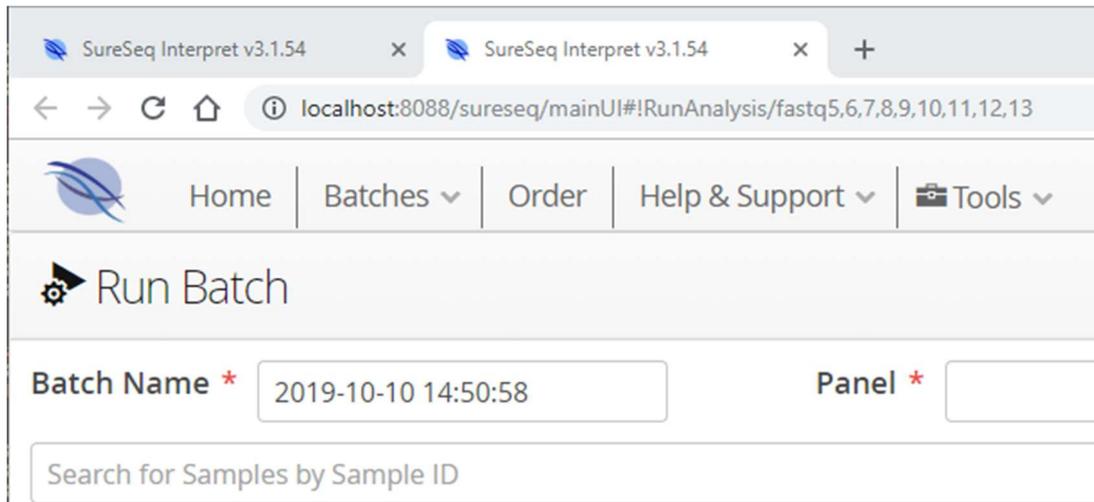


Figure 1: The **Upload FASTQs** tab is hidden behind the new tab in Chrome. Uploading is paused.

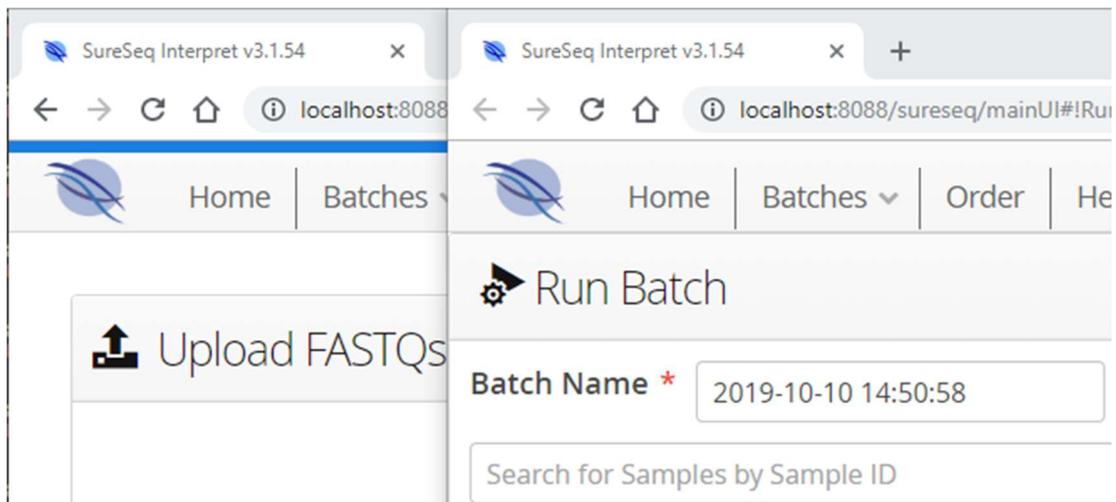


Figure 2: The new tab is separated from the **Upload FASTQs** tab. Uploading will continue in the background.

Start the Batch

Home | Batches | Order | Help & Support | Tools v3.1.54

Run Batch

Batch Name * My First Batch Panel * CytoSure NGS Panel Protocol * Default Protocol

Search for Samples by Sample ID

ID	Sample ID	FASTQ Read 1	FASTQ Read 2
13	24315	24315_7_L000_R1.fastq.gz	24315_7_L000_R2.fastq.gz
12	12878	12878_7_L000_R1.fastq.gz	12878_7_L000_R2.fastq.gz
11	11516	11516_7_L000_R1.fastq.gz	11516_7_L000_R2.fastq.gz
10	10847	10847_7_L000_R1.fastq.gz	10847_7_L000_R2.fastq.gz

Selected Samples (9)*

ID	Sample ID	FASTQ Read 1	FASTQ Read 2
5	156	156_R1.fastq.gz	156_R2.fastq.gz
6	289	289_R1.fastq.gz	289_R2.fastq.gz
7	4315	4315_7_L000_R1.fastq.gz	4315_7_L000_R2.fastq.gz
8	6937	6937_6_L001_R1_001.fastq.gz	6937_6_L001_R2_001.fastq.gz
9	10384	10384_7_L000_R1.fastq.gz	10384_7_L000_R2.fastq.gz
10	10847	10847_7_L000_R1.fastq.gz	10847_7_L000_R2.fastq.gz

→ Run Analysis

- The samples previously selected for the batch should be listed in the **Selected Samples** table. If they are not, select the correct samples from the table above.
 - Enter an appropriate name for the batch in the **Batch Name** field.
 - Select the appropriate panel from the **Panel** drop-down box.
 - Select **Default Protocol** from the **Protocol** drop-down box.
 - Click **Run Analysis**.

Are you sure you would like to start the following Analysis?

Name My First Batch

SureSeq Panel CytoSure NGS Panel

Protocol Default Protocol

Samples (9)

ID	Sample ID	FASTQ Read 1	FASTQ Read 2
5	156	156_R1.fastq.gz	156_R2.fastq.gz
6	289	289_R1.fastq.gz	289_R2.fastq.gz
7	4315	4315_7_L000_R1.fastq.gz	4315_7_L000_R2.fastq.gz
8	6937	6937_6_L001_R1_001.fastq.gz	6937_6_L001_R2_001.fastq.gz
9	10384	10384_7_L000_R1.fastq.gz	10384_7_L000_R2.fastq.gz
10	10847	10847_7_L000_R1.fastq.gz	10847_7_L000_R2.fastq.gz

Cancel OK

- Click **OK** to confirm that the details are correct and start the batch.

Monitor the Batch

The **Batch** view provides information on the progress of the analysis pipeline as it processes the samples in the batch. The status of both the batch as a whole and its individual samples is updated during processing so that progress can be monitored by the user.

Completed Samples

Sample	# SNVs	# CNVs	# LOH	% Reads Aligned	% Duplication	% Usable On Target Reads	% Usable On And Near Target Reads	% Reads Mapping Quality
NA10384	2,751	59	17	99.53	8.36	73.19	82.7	2.94
NA10847	2,766	81	14	99.46	8.29	72.07	80.35	3.41
NA18537	2,794	50	21	99.54	8.78	73.36	82.52	2.9
NA18542	2,729	36	21	99.51	7.81	74.19	83.42	3.0
NA18939	2,762	58	21	99.53	9.09	73.28	82.88	2.87
NA19022	3,446	45	6	99.52	8.18	72.66	82.7	3.08
NA19671	2,830	28	14	99.53	8.9	73.14	82.7	3.01
NA19923	3,220	70	8	99.48	5.87	72.75	81.42	3.15
NA20508	2,717	41	21	99.5	8.28	72.25	81.9	2.85
NA20514	2,721	26	15	99.54	7.77	72.23	81.89	3.11

The batch may be assigned the following statuses as it progresses, which are displayed in the **Status** field in the **Overview** section:

- **Waiting** - Waiting for the batch to be queued on the processing server, or for another batch to complete.
- **Transferring FASTQs** - Transferring the FASTQs of the test samples to the processing server.
- **Pre-processing Reference Samples** - Transferring and aligning the FASTQ files of the reference samples defined in the analysis protocol. The progress of each individual reference sample is also displayed.
- **In progress** - Running the pipeline on test samples.
- **Completed** - All samples have been processed successfully.
- **Failed** - The batch failed for at least one sample.
- **Killed** - The user forcibly stopped the batch.

If the batch doesn't appear to be progressing, or has failed, the **File Status** page provides the ability to download the logs for an individual sample which will help technical support with troubleshooting.

As each sample completes, an entry will appear in the **Completed Samples** table containing a summary of its QC metrics and variants detected.

View the QC Metrics

The **Batch** view provides access to QC metrics for the samples as follows:

1. In the **Completed Samples** table - The values of a number of QC metrics are presented to the user in the **Completed Samples** table. Some may be hidden from the table by default, but can be displayed by clicking on the  icon in the top-right corner of the table and selecting the missing columns.
2. In the "fastp" report - The **QC** column of the **Completed Samples** table provides access to the report generated by the "fastp" utility via the **View** button.
3. In the "MultiQC" report - The **Overview** section contains a link to the **MultiQC Report**, which includes various metrics and graphs of metrics for every sample in the batch in one HTML document.

View the CNV/LOH Data

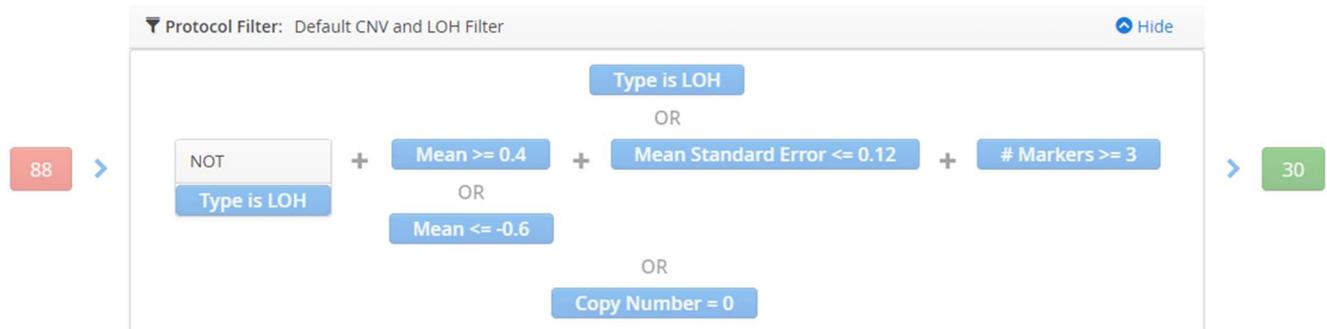
For selected, view:

SNVs/Indels

CNVs/LOH Calls

Completed Samples		Sample	# SNVs	# CNVs	# LOH	% Reads Aligned
<input type="checkbox"/>	View	NA10384	2,751	59	17	99.53
<input checked="" type="checkbox"/>	SNVs CNVs/LOH VCF Logs	NA10847	2,766	81	14	99.48
<input type="checkbox"/>	SNVs CNVs/LOH VCF Logs	NA18537	2,794	50	21	99.54
<input checked="" type="checkbox"/>	SNVs CNVs/LOH VCF Logs	NA18542	2,729	36	21	99.51
<input type="checkbox"/>	SNVs CNVs/LOH VCF Logs	NA18939	2,762	58	21	99.53
<input checked="" type="checkbox"/>	SNVs CNVs/LOH VCF Logs	NA19022	3,446	45	6	99.52
<input checked="" type="checkbox"/>	SNVs CNVs/LOH VCF Logs	NA19671	2,830	28	14	99.53
<input checked="" type="checkbox"/>	SNVs CNVs/LOH VCF Logs	NA19923	3,220	70	8	99.48
<input checked="" type="checkbox"/>	SNVs CNVs/LOH VCF Logs	NA20508	2,717	41	21	99.5
<input checked="" type="checkbox"/>	SNVs CNVs/LOH VCF Logs	NA20514	2,721	26	15	99.54

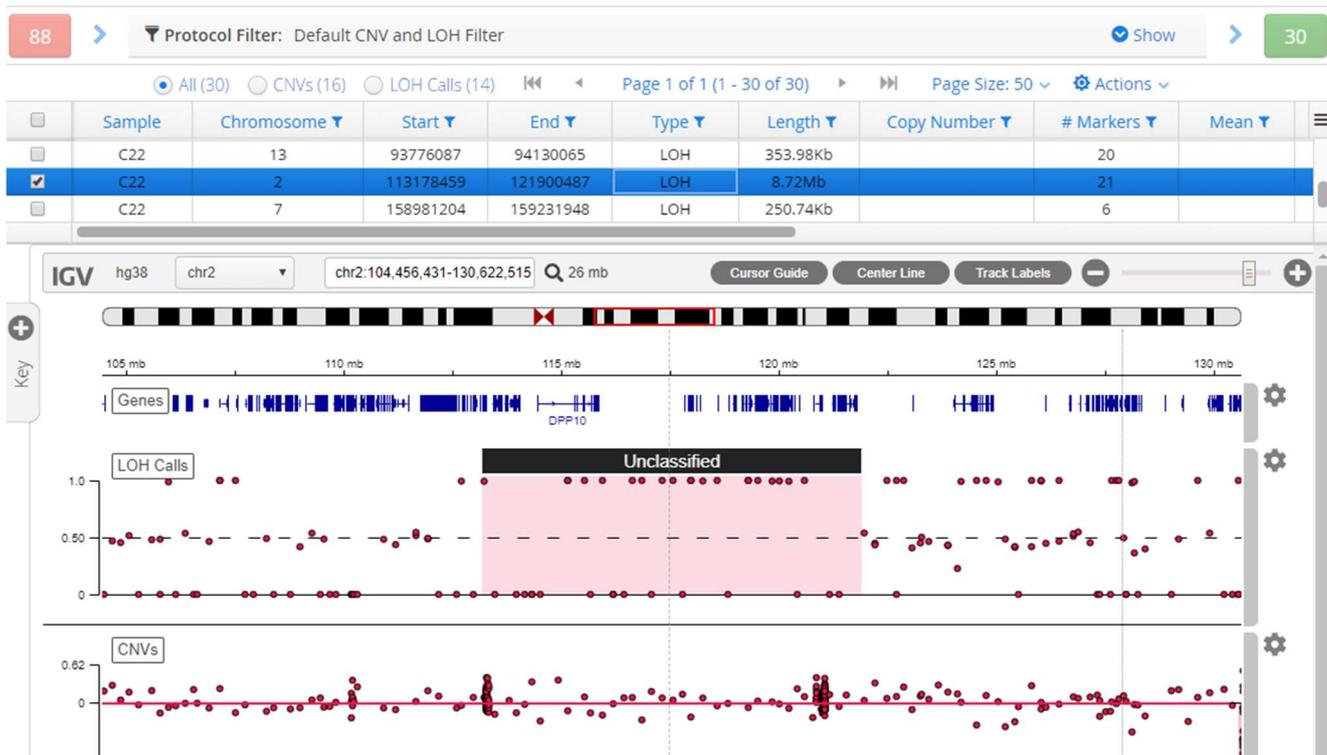
1. To view CNV/LOH data for a set of samples, select the corresponding checkbox for the sample in the **Completed Samples** table and click the **CNVs/LOH Calls** button. Alternatively, to view CNV/LOH data for an individual sample, click the **CNVs/LOH** in the **View** column for the sample.



2. The **CNVs + LOH Calls** view should appear. The **Protocol Filter** section at the top displays the filters which have been applied to the detected CNVs and LOH Calls. This can be minimised by clicking the **Hide** button.

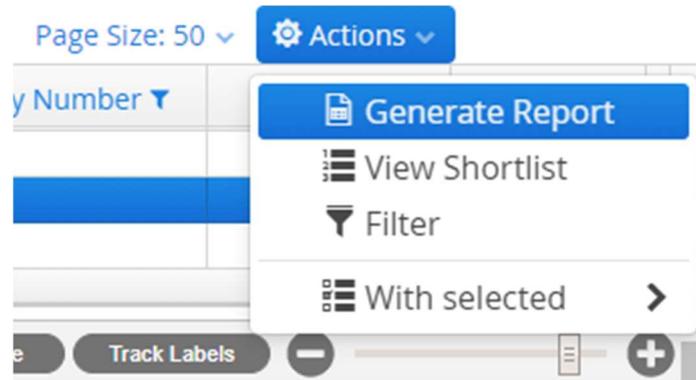
Sample	Chromosome	Start	End	Type	Length	Copy Number	# Markers	Mean	Confidence	Overlap	Classification	Depth	Frequency	Mean Standard Error
C22	2	44297436	44850269	LOH	552.83Kb		19				Unclassified			
C22	21	43168997	43169247	Deletion	250b	0	5	-6.56238	High	CDS (target)	Unclassified	1		0.52303
C22	19	48442590	48443190	Duplication	600b	3	12	0.504963	High	CDS (target)	Unclassified	202	0.48	0.0471814
C22	7	130476747	130838709	LOH	361.96Kb		17				Unclassified			
C22	Y	3491379	10095513	LOH	6.6Mb		4				Unclassified			
C22	18	74586359	75294325	LOH	707.97Kb		14				Unclassified			
C22	17	46170855	46225432	Duplication	54.58Kb	4	43	0.81565	High	CDS (target)	Unclassified	1512	0.48	0.0149918
C22	3	177197121	177197341	Duplication	220b	3	4	0.55892	High	no CDS	Unclassified	179		0.0426823
C22	3	130603170	130603630	Duplication	460b	1	0	0.48833	High	no CDS	Unclassified	333	0.48	0.0408773

3. The CNVs and LOH Calls detected in the selected sample(s) are listed in the table in the middle of the page.

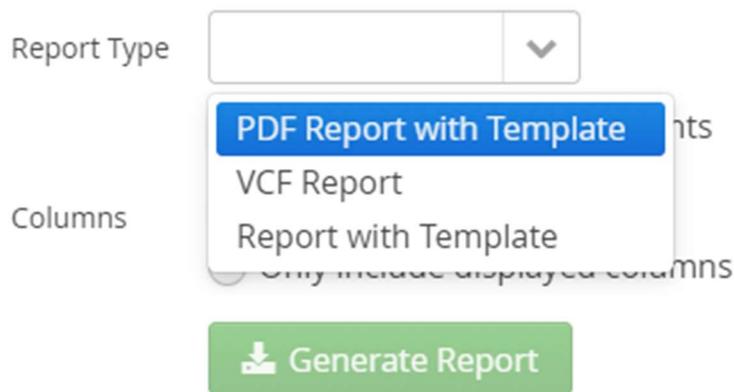


4. To view the genomic data contributing to a CNV or LOH call, click on the corresponding row of the table and the **IGV** section will be populated accordingly.

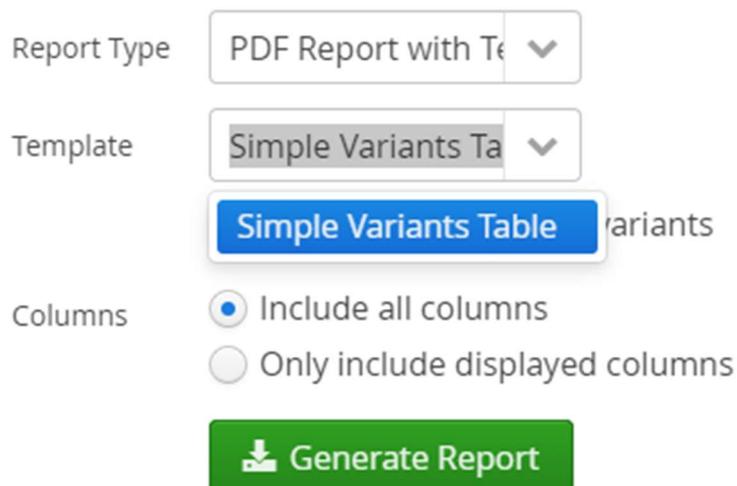
Generate a Report of the CNV/LOH Data



1. Select the **Actions** button above the CNV/LOH Calls table and click **Generate Report**.



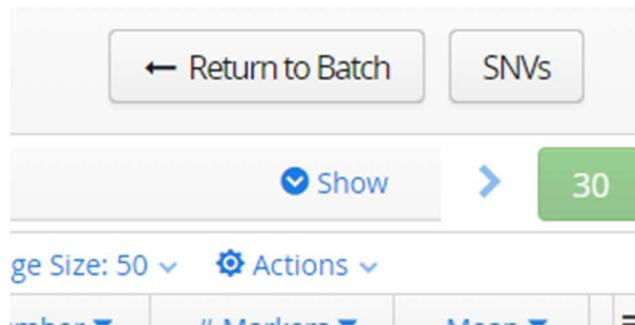
2. In the **Report Type** drop-down list, select the type of report you wish to generate.



3. If necessary, in the **Template** drop-down list, select a report template.

4. Choose whether to **Include all columns** or **Only include displayed columns** and click **Generate Report**.

View the SNV Data



1. To view SNV data for the sample(s) currently displayed in the **CNVs + LOH Calls** view, click the **SNVs** button in the top-right corner.

For selected, view:

Completed Samples

<input type="checkbox"/>	View	Sample	# SNVs	# CNVs	# LOH	% Reads Aligned
<input type="checkbox"/>	<input type="button" value="SNVs"/> <input type="button" value="CNVs/LOH"/> <input type="button" value="VCF"/> <input type="button" value="Logs"/>	NA10384	2,751	59	17	99.53
<input checked="" type="checkbox"/>	<input type="button" value="SNVs"/> <input type="button" value="CNVs/LOH"/> <input type="button" value="VCF"/> <input type="button" value="Logs"/>	NA10847	2,766	81	14	99.46
<input type="checkbox"/>	<input type="button" value="SNVs"/> <input type="button" value="CNVs/LOH"/> <input type="button" value="VCF"/> <input type="button" value="Logs"/>	NA18537	2,794	50	21	99.54
<input checked="" type="checkbox"/>	<input type="button" value="SNVs"/> <input type="button" value="CNVs/LOH"/> <input type="button" value="VCF"/> <input type="button" value="Logs"/>	NA18542	2,729	36	21	99.51
<input type="checkbox"/>	<input type="button" value="SNVs"/> <input type="button" value="CNVs/LOH"/> <input type="button" value="VCF"/> <input type="button" value="Logs"/>	NA18939	2,762	58	21	99.53
<input checked="" type="checkbox"/>	<input type="button" value="SNVs"/> <input type="button" value="CNVs/LOH"/> <input type="button" value="VCF"/> <input type="button" value="Logs"/>	NA19022	3,446	45	6	99.52
<input checked="" type="checkbox"/>	<input type="button" value="SNVs"/> <input type="button" value="CNVs/LOH"/> <input type="button" value="VCF"/> <input type="button" value="Logs"/>	NA19671	2,830	28	14	99.53
<input checked="" type="checkbox"/>	<input type="button" value="SNVs"/> <input type="button" value="CNVs/LOH"/> <input type="button" value="VCF"/> <input type="button" value="Logs"/>	NA19923	3,220	70	8	99.48
<input checked="" type="checkbox"/>	<input type="button" value="SNVs"/> <input type="button" value="CNVs/LOH"/> <input type="button" value="VCF"/> <input type="button" value="Logs"/>	NA20508	2,717	41	21	99.5
<input checked="" type="checkbox"/>	<input type="button" value="SNVs"/> <input type="button" value="CNVs/LOH"/> <input type="button" value="VCF"/> <input type="button" value="Logs"/>	NA20514	2,721	26	15	99.54

Alternatively, click **Return to Batch**, select the sample(s) from the **Completed Samples** table and click the **SNVs/Indels** button.

Protocol Filter: Chain of 4 filters Hide

2784 >

 +

 +

 +

 >
 2698

2. The **Variants** view should appear. As with the **CNVs + LOH Calls** view, the **Protocol Filter** section at the top of the page displays the filters which have been applied to the detected SNVs/Indels, which can be hidden by clicking the **Hide** button.

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<input type="checkbox"/>	HGVSc (Gene Symbol)	Chromosome	Start	End	Ref	Alt	Allele Frequency	Type	Ref Depth
<input type="checkbox"/>	DVL1:c.*347T>C	1	1335795	133579	A	G	100%	SNV	0
<input type="checkbox"/>	DVL1:c.366A>G	1	1342153	134215	T	C	100%	SNV	0
<input type="checkbox"/>	DVL1:c.-45C>T	1	1349110	134911	G	A	100%	SNV	0
<input type="checkbox"/>	ATAD3A:c.*94G>A	1	1534166	153416	G	A	99.6%	SNV	1
<input type="checkbox"/>	SKI:c.*1802T>C	1	2308567	230856	T	C	99.45%	SNV	3
<input type="checkbox"/>	SKI:c.*3182dup	1	2309937	230993	C	CT	82.96%	Insertion	23
<input type="checkbox"/>	SKI:c.*3181_*3182dup	1	2309937	230993	C	CTT	75.53%	Insertion	23
<input type="checkbox"/>	CAMTA1:c.*580T>C	1	6888144	688814	T	C	99.8%	SNV	1
<input type="checkbox"/>	CAMTA1:c.1350G>A	1	7663897	766389	G	A	48.32%	SNV	293
<input type="checkbox"/>	CAMTA1:c.*1364_*1365dup	1	7767843	776784	C	CAA	92.95%	Insertion	11

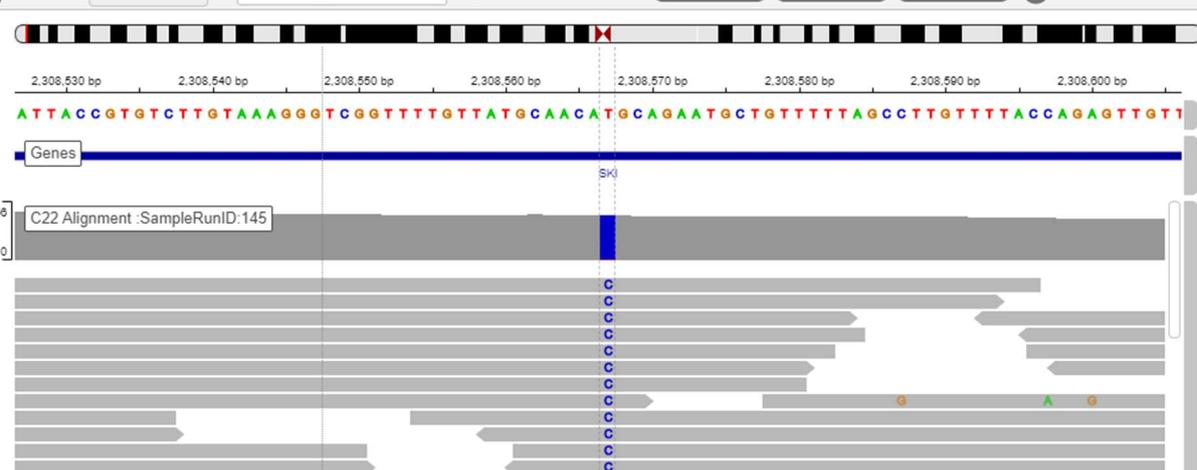
3. The table in the middle of the page lists the SNVs/Indels detected in the selected sample(s). Additional information is available for display via the  button.

Page 1 of 270 (1 - 10 of 2698) Page Size: 10 Actions

<input type="checkbox"/>	HGVSc (Gene Symbol)	Chromosome	Start	End	Ref	Alt	Allele Frequency	Type	Ref Depth
<input type="checkbox"/>	DVL1:c.-45C>T	1	1349110	134911	G	A	100%	SNV	0
<input type="checkbox"/>	ATAD3A:c.*94G>A	1	1534166	153416	G	A	99.6%	SNV	1
<input checked="" type="checkbox"/>	SKI:c.*1802T>C	1	2308567	230856	T	C	99.45%	SNV	3
<input type="checkbox"/>	SKI:c.*3182dup	1	2309937	230993	C	CT	82.96%	Insertion	23

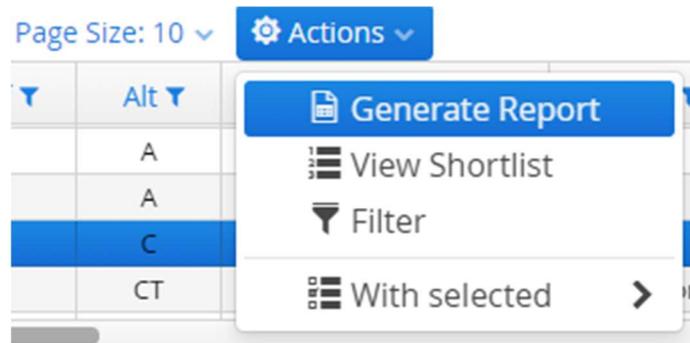
IGV hg38 chr1 chr1:2,308,527-2,308,607 81 bp

Cursor Guide Center Line Track Labels

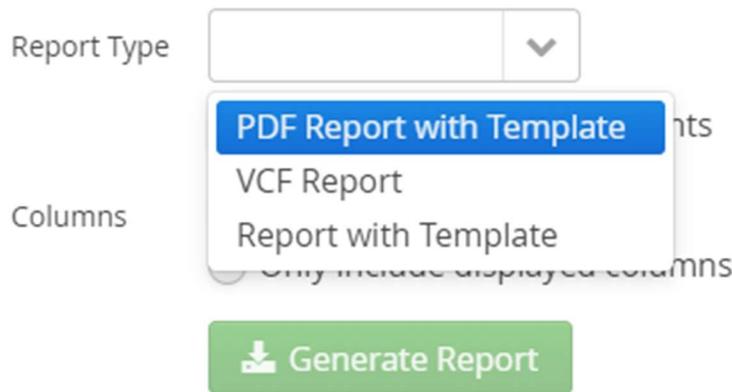


4. To view the genomic data contributing to a SNV or Indel, click on the corresponding row of the table and the **IGV** section will be populated accordingly,

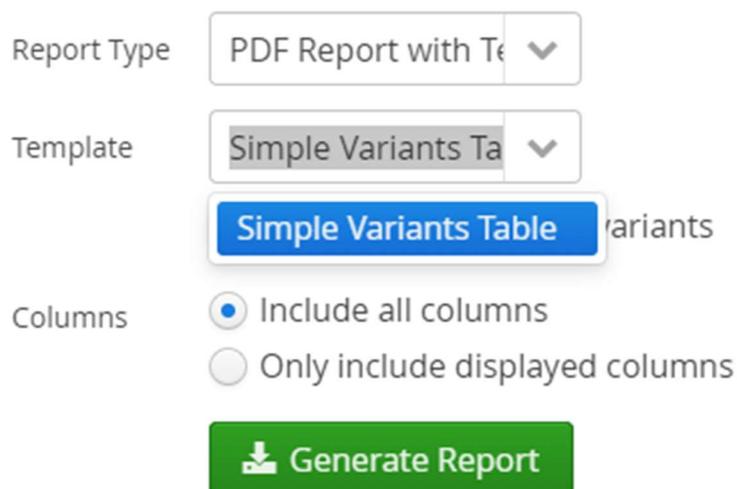
Generate a Report of the SNV Data



1. Select the **Actions** button above the SNVs/Indels table and click **Generate Report**.



2. In the **Report Type** drop-down list, select the type of report you wish to generate.



3. If necessary, In the **Template** drop-down list, select a report template.

4. Choose whether to **Include all columns** or **Only include displayed columns** and click **Generate Report**.

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