



Tutorial

Find Actionable Variants With QIAseq DNA Panels

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— Sample to Insight —

Find Actionable Variants With QIAseq DNA Panels

This tutorial uses the capabilities of the CLC Genomics Workbench and Biomedical Genomics Analysis plugin to find actionable variants - even at very low frequencies - in Targeted Amplicons sequences generated using a BRCA1 and BRCA2 QIAseq panel kit.

This tutorial covers in just a few steps all the following:

- Import Illumina paired reads in the workbench.
- Find low frequency variants with the **Analyze QIAseq Panels** guide.

Prerequisites For this tutorial, you must be working with CLC Genomics Workbench 12.0. You must also have installed the Biomedical Genomics Analysis plugin. How to install plugins is described here: <http://resources.qiagenbioinformatics.com/manuals/clcgenomicsworkbench/current/index.php?manual=Install.html>. For the last section, Upload your results to QCI Interpret, you need an active QCI Interpret subscription (not a trial license), and a QCI Interpret account with API license privileges.

Import the reads

The Analyze QIAseq Panels guide will help you run a panel analysis workflow in a streamlined way.

1. First, download the sample data from our website:

http://resources.qiagenbioinformatics.com/testdata/QIAseq_DNA_tutorial.zip

and unzip it in the location of your choice on your computer. Note that the folder contains more files than you will need to use, but that can be useful if you are working with older versions of the workbench and plugin.

2. Start the *CLC Genomics Workbench*.
3. Open the Analyze QIAseq Panels guide here:

Ready-to-Use Workflows | QIAseq Panel Analysis | Analyze QIAseq Panels

4. Click on **Import Illumina reads**, select the two fastq files you just saved on your computer, and set up the importer configuration as seen in figure 1: all parameters remain as they are set by default, except for "Paired-end reads" that needs to be checked.
5. Click **Next** and choose the folder you wish to save the reads to (you can create a new folder dedicated to this tutorial for example) before clicking **Finish**.

Both files will be merged as a single file of paired reads during import.

Run the Analyze QIAseq Panels guide

We will now run the BRCA1 and BRCA2 Panel (DHS-102Z) workflow with all the settings set to their default values.

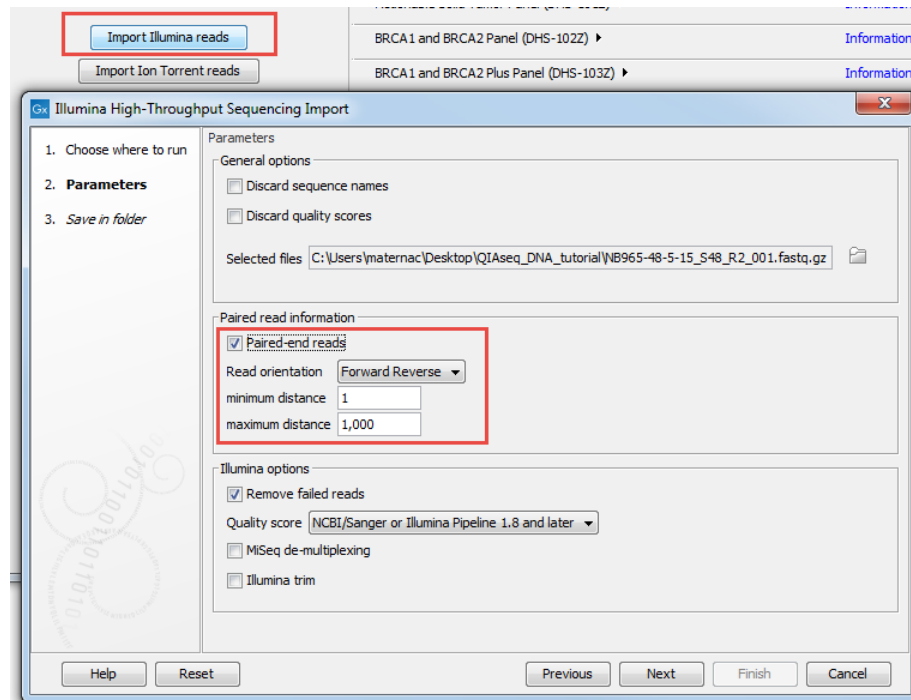


Figure 1: Import the reads.

1. Click on the the arrow to the right of the BRCA1 and BRCA2 Panel (DHS-102Z) workflow, select the option **Somatic, Illumina**, and click **Run**. If you are connected to a Server, you will be asked where to run the workflow. We recommend that you choose the Server when possible.
2. The first dialog will require for you to acquire the relevant reference data set (figure 2). Click on **Download to Workbench**, then on **Next**.

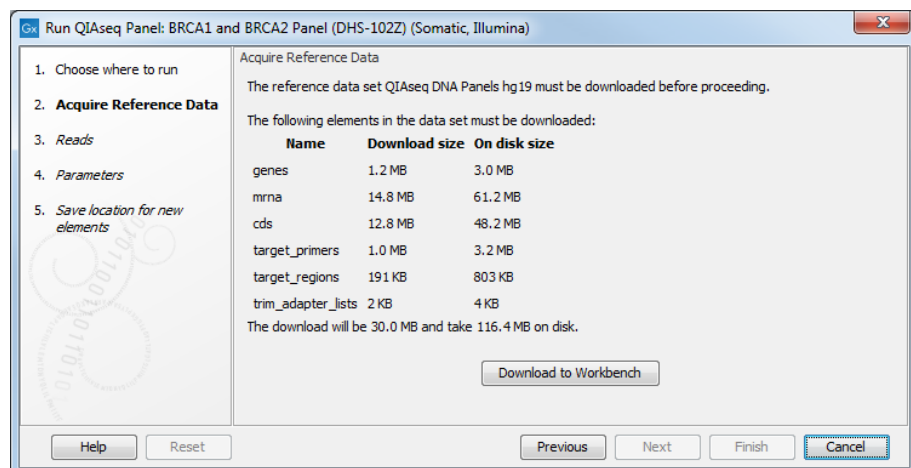


Figure 2: Acquire reference data set by clicking Download to Workbench.

3. Select the sequencing reads that should be analyzed (figure 3) and click **Next**.
4. In the next dialog (figure 4), leave all parameters as they are set by default for somatic applications and click **Next**.

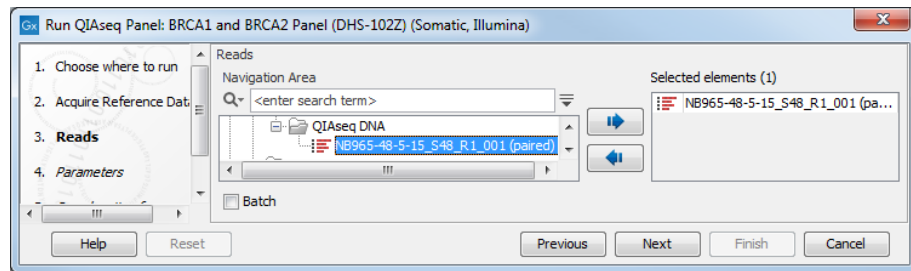


Figure 3: After clicking Run, select the sequencing reads by double-clicking on the file name or by clicking once on the file name and then on the arrow pointing to the right hand side.

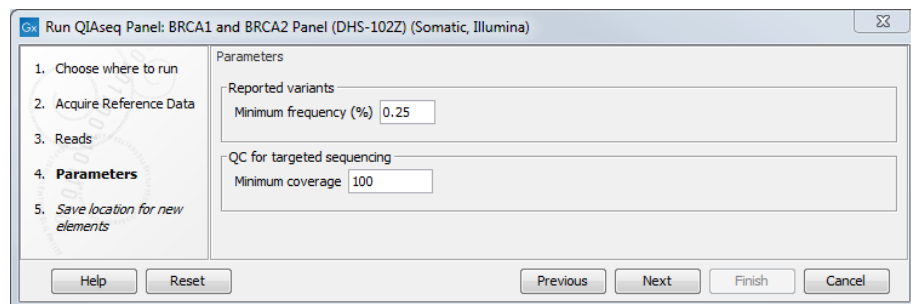






Figure 4: Leave all parameters as set by default as they have been optimized for somatic variants detection.

5. Finally, in the last wizard step, choose to **Save** the results of the workflow and specify a location in the Navigation Area before clicking **Finish**.

Output from the BRCA1 and BRCA2 Panel Analysis

The BRCA1 and BRCA2 Panel Analysis workflow produces the following outputs (most of which are available in a subfolder as seen in figure 5):

- a track List 
- a Trim Reads Report 
- a UMI Groups Report and a Create UMI Report 
- a read mapping of the UMI Reads 
- a coverage report and a coverage graph
- three variant tracks: the Unfiltered Variants is output before the filtering steps, the Variants passing filters is the one used in the Genome Browser View. The Amino acid track allows to distinguish variants responsible for changes at the amino acid level.

First, it is very important that the quality requirements are met to use QIAseq DNA panels workflows. Learn about Quality Control for the Identify QIAseq DNA Variants workflow in the manual: http://resources.qiagenbioinformatics.com/manuals/biomedicalgenomicsanalysis/current/index.php?manual=Quality_Control_Identify_QIAseq_DNA_Variants_workflow.html.

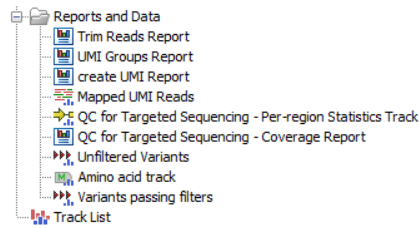


Figure 5: Output from the BRCA1 and BRCA2 Panel Analysis workflow.

Then double click on the Track List output. The file opens as a split screen with both Track List and the corresponding variant table. In the table, filter for variants for which the allele is not identical to the reference: open the advanced filter by clicking on the arrow in the top right corner of the table. Fill in the filtering fields with "**Reference allele**", "=", and type "**No**" (figure 6).

QIAseq panels allow the detection of very low frequency variants. To observe this is in the tutorial data, sort the "**Frequency**" column in the variant table in ascending order by clicking on the header of that column. Variants with a frequency ("Count" divided by "Coverage") even lower than 2 appear at the top of the table.

Clicking on a particular row in the variant table causes the Genome Browser to zoom in to the nucleotide level with the focus on that variant position. This allows you to view the coverage and the context of this variant, e.g., Mapped UMI Reads, Amino acid track, and so on.

Upload your results to QCI Interpret

It is now possible to upload one of the variant tracks to QCI Interpret. For this section, you need an active QCI Interpret subscription (not a trial license), and a QCI Interpret account with API license privileges.

1. To start the upload, go to:

Tools | QIAseq Panel Expert Tools | QCI Interpret Integration | Upload to QCI Interpret

2. In the first dialog (figure 7), select a variant track from which a VCF will be uploaded to QCI Interpret. In addition, select the reference sequence (it is set as hg19 by default, but hg38 is also supported). You can then choose which application you would like for the QCI Interpret results (Somatic or Hereditary).
3. In the Server Settings dialog (figure 8), fill in the field with the following information:
 - Server location. QCI Interpret is hosted in multiple locations. Your QCI Interpret account is created for a specific QCI Interpret server that should be specified here.
 - API key ID and API key secret. If you do not know your API credentials, contact support-license@qiagen.com.
 - Username. Insert the username associated with the QCI Interpret account.
4. It is then possible to set up filters to reduce the amount of variants to send to QCI Interpret (figure 9). In this tutorial, we choose to leave all filters at their default value, i.e., we do not set any filter.



Figure 6: Track List.

Clicking on **Finish** only initiate the transfer of variants or fusions to QCI Interpret: the tool will open a browser window with the QCI Interpret interview page. This page must be filled in and the button **Submit** must be pressed to complete the upload of the files and metadata to QCI Interpret. In cases where a browser window fails to open automatically, the tool will also output a report containing the link to the QCI Interpret interview page (figure 10).

Please refer to the QCI Interpret documentation to learn more about the interpretation of the results (figure 11).

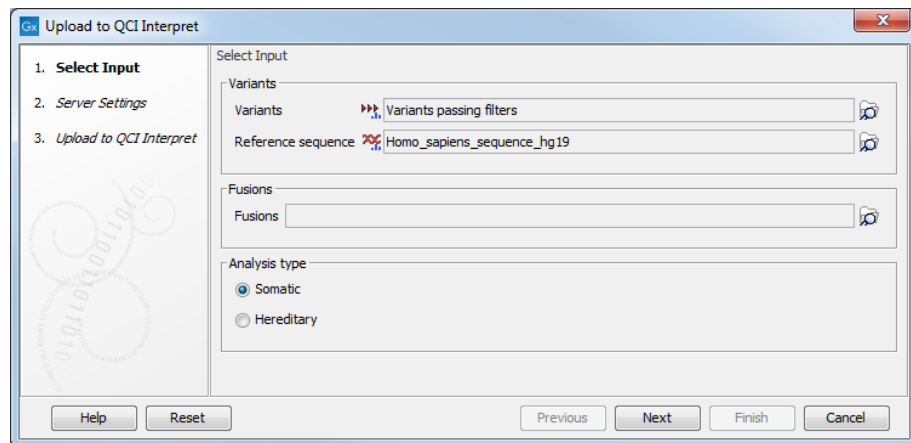


Figure 7: Select the variants file you would like to interpret.

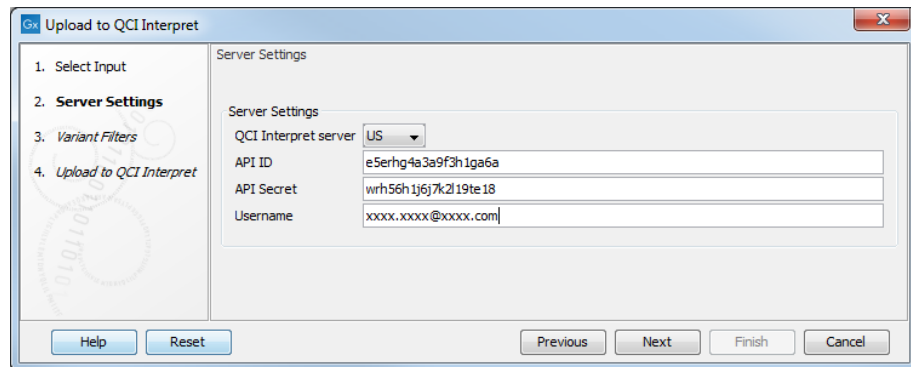


Figure 8: Configure the connection to the QCI Interpret server dialog.

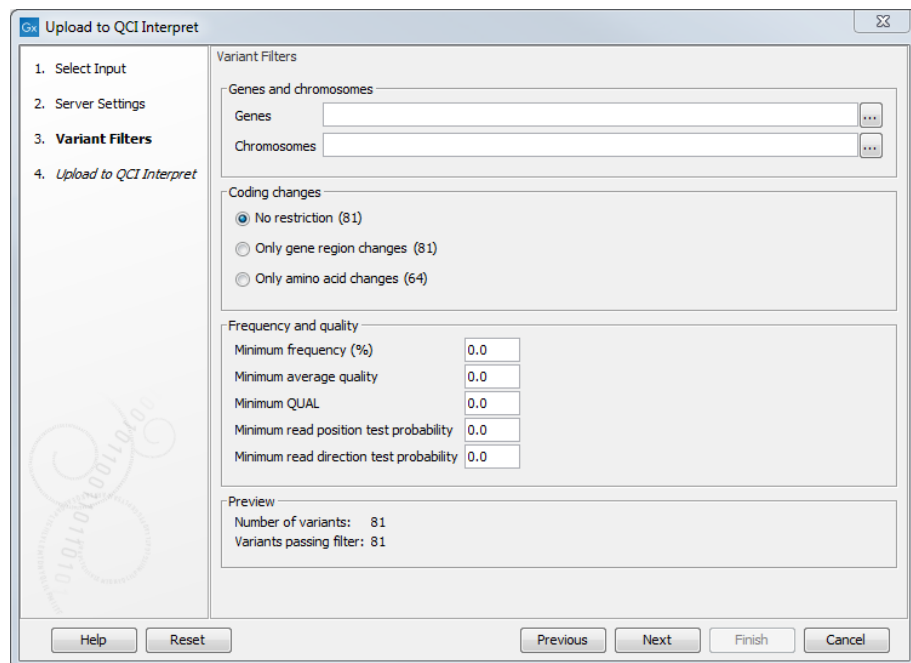


Figure 9: Filters can be set to reduce the amount of variants sent to interpretation.

1 Results

The analysis has accession id qiaseq-panel-upload-18052

After filling out the interview page the result of the analysis will be at:

https://api.ingenuity.com/datastream/analysisStatus.jsp?packageId=DP_1053

2 Warnings

QCI Interpret reported the following warning

- We could not detect "AD" in the FORMAT field of qiaseq-panel-upload-180525-105148.vcf. It is recommended that you supply depth of coverage for REF/ALT alleles in order to

3 Settings

QCI Interpret username	qcia-	@qiagen.com
QCI Interpret environment	US	

For further details consult the history of this report.

4 Interview page

A browser should have been launched and redirected to the QCI Interpret interview page. Otherwise it can be accessed via the below link:

<file:///C:/Users/maternac/AppData/Local/Temp/qcilauncher63.html>

Note that the above page is temporary and stored locally. It may be deleted after some time, including when closing the workbench.

Figure 10: Filters can be set to reduce the amount of variants sent to interpretation.

The screenshot shows the QCI Interpret website interface. At the top, there's a navigation bar with 'Clinical Insight', 'Variant List', 'Variant Detail', and 'Review & Report'. The main header displays 'Accession ID (Test Product Code) qiaseq-panel-upload-7891' and 'Diagnosis cancer'. Below this, a detailed view of a variant is shown: Gene BRCA2, Variant c.2590C>T p.Q864*, Somatic Frequency: 0%, Population Frequency: 0% gnomAD, Allele Fraction: 1.84% (of 488 reads), Impact: stop gain, and Computed Classification: Tier 1A Pathogenic Cancer. A grid of 32 variant cards follows, each representing a different variant with its gene, variant ID, and classification. A sidebar on the right contains filters for Status (To be assessed (124), Assessed), Actionability (Tier 1A/1B, Tier 3, Tier 4, Clinical Trials, Prognostic), Pathogenicity (Pathogenic, Likely Pathogenic, Uncertain Significance, Likely Benign, Benign, Conflicting Criteria), and Origin (Likely Somatic, Likely Germline).

Figure 11: Results of the interpretation analysis from the QCI Interpret website.