



# Tutorial

## Find Actionable Variants With QIAseq DNA Panels

November 21, 2017

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— Sample to Insight —

## Find Actionable Variants With QIAseq DNA Panels

This tutorial uses the capacities of the Biomedical Genomics Workbench and the QIAseq Targeted Panel 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:

- Download the relevant Reference Data Set.
- Import Illumina paired reads in the workbench.
- Set up trim adapter lists to remove the sequencing primers off the reads.
- Find low frequency variants with the **Analyze QIAseq Panels** guide.

### Prerequisites

For this tutorial, you must be working with the Biomedical Genomics Workbench 5.0 or higher. You must also have installed the QIAseq Targeted Panel Analysis Plugin 1.0. How to install plugins is described here: [http://resources.qiagenbioinformatics.com/manuals/biomedicalgenomicsworkbench/current/index.php?manual=Installing\\_plugins.html](http://resources.qiagenbioinformatics.com/manuals/biomedicalgenomicsworkbench/current/index.php?manual=Installing_plugins.html).

### Data management configuration

In order to do this tutorial, you need to use the QIAseq DNA Panels hg19 Reference Data Set.

1. To download this dataset, go to:

**Toolbar | Data Management** (📁)

2. This will open the wizard shown in figure 1.
3. If you are connected to a CLC Server, choose to download the reference on the Server instead of locally.
4. Select the Reference Data Set QIAseq DNA Panels hg19 and click on the button labeled **Download**.
5. Once the download is complete, you can now close the Data Manager.

### 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](http://resources.qiagenbioinformatics.com/testdata/QIAseq_DNA_tutorial.zip)

and unzip it in the location of your choice on your computer.

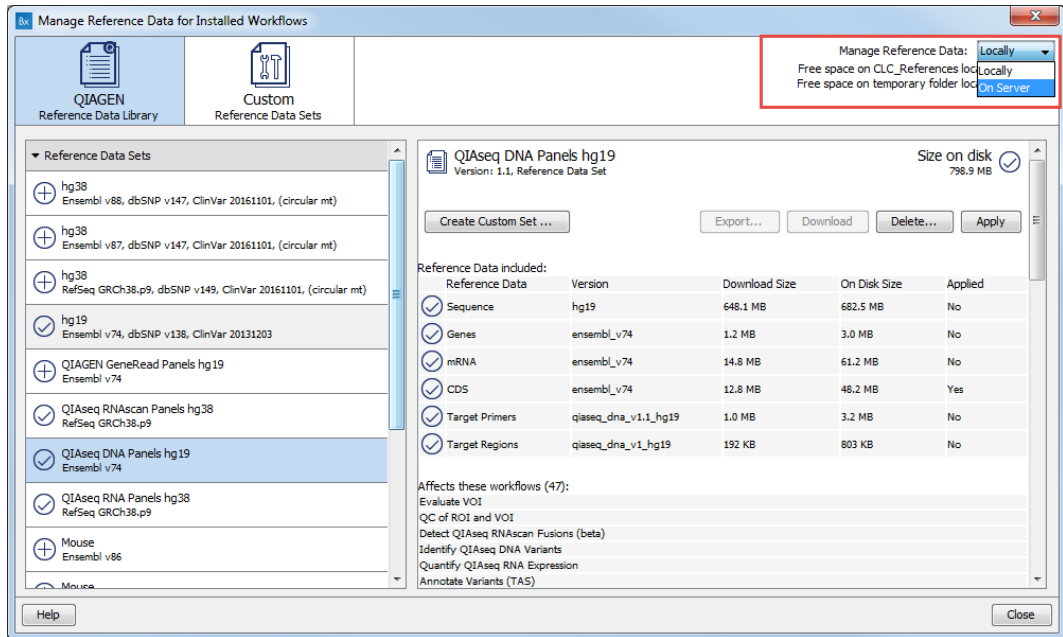


Figure 1: Open the Data Management and download QIaseq DNA Panels hg19 Reference Data Set.

2. Start the *Biomedical Genomics Workbench*.
3. Open the Analyze QIaseq Panels guide here: **Ready-to-Use Workflows | QIaseq Panel Analysis**
4. Click on **Import sequencing data**, select the two fastq files you just saved on your computer, and set up the importer configuration as seen in figure 2: all parameters remain as they are set by default, except for "Paired-end reads" that needs to be checked.

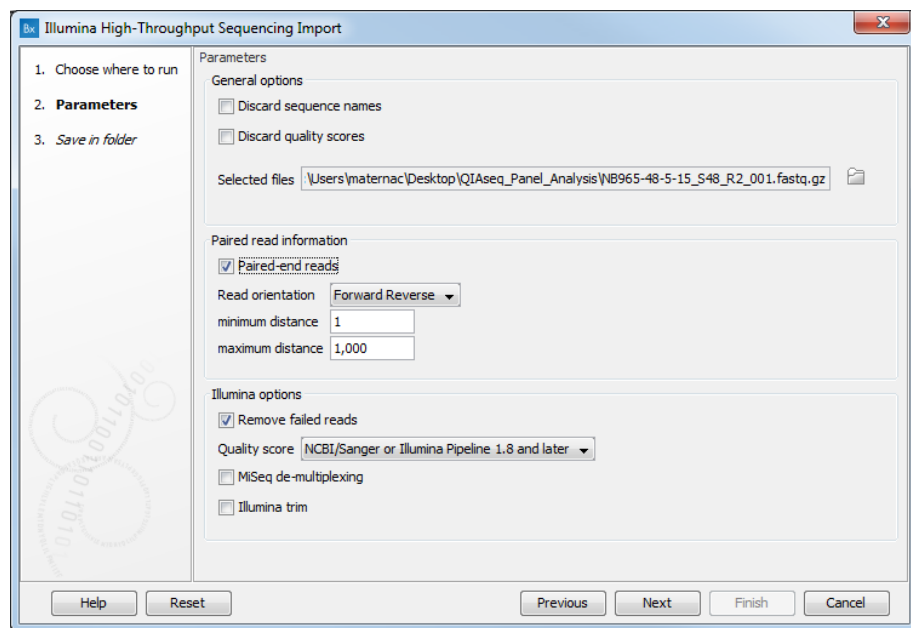


Figure 2: Import the reads.

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.

### Set up the trim adapter list

Use the Analyze QIAseq Panels guide to add the missing oligonucleotide sequences.

1. Click on Set up adapters (figure 3).

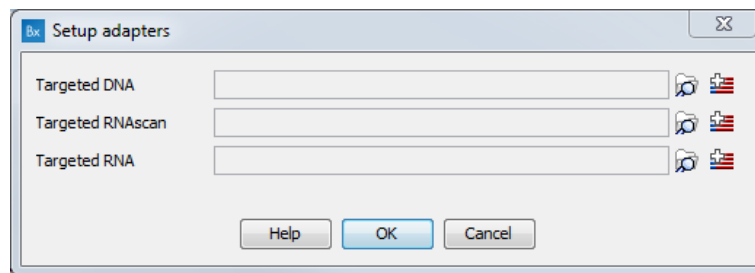


Figure 3: Application-specific trim adapter lists.

2. Click on the Trim adapter list button to the right of the Targeted DNA field.
3. Then follow the instructions given in the dialog, i.e., insert the Nextera transposase adapter sequences (Read 1 and Read 2) by copying them from page 12 of the Illumina Customer Sequence Letter found at this address: <http://support.illumina.com/downloads/illumina-customer-sequence-letter.html>.<sup>1</sup>  
 Read 1 (5'-3'): copy and paste here Nextera Transposase Adapter Read 1 sequence  
 Read 2 (5'-3'): copy and paste here Nextera Transposase Adapter Read 2 sequence
4. Click Next, and select a location in the Navigation Area where you want the trim adapter list to be saved before clicking Finish. Remember to save the list on the server if this is where you intend to run the workflows.
5. In the Set up adapters window, click on the Browse button and select the Trim adapter list you just saved.

### How to 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.

1. Click on the the Run button to the right of the DHS-102Z workflow.
2. 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.
3. Select the sequencing reads that should be analyzed (figure 4) and click **Next**.

<sup>1</sup>Oligonucleotide sequences © 2007-2013 Illumina, Inc. All rights reserved. No sponsorship or affiliation. Link provided for convenience. QIAGEN not responsible for content at link.

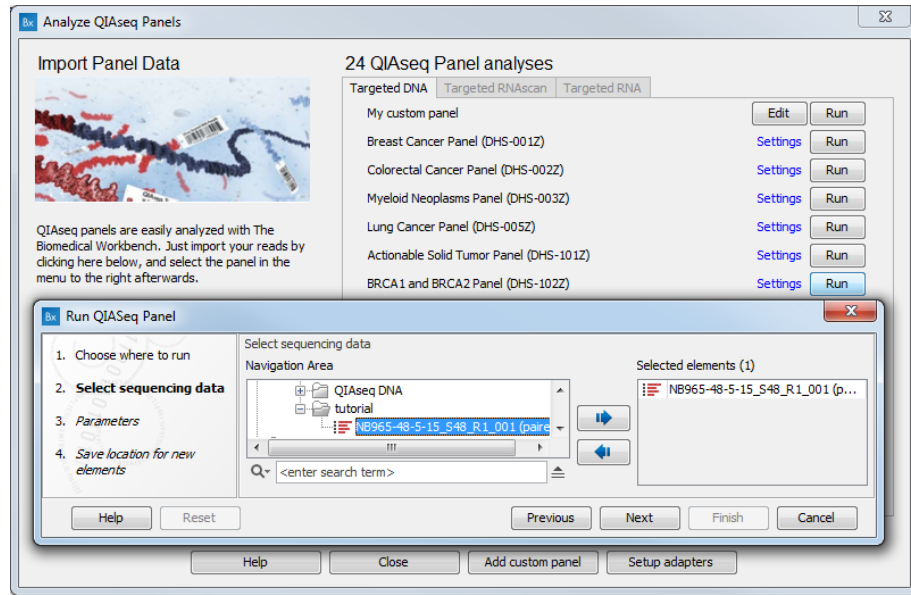


Figure 4: After clicking run, select the sequencing reads by double-clicking on the file name or by clicking once on the file name and then on the arrow pointing to the right hand side.

4. In the next dialog (figure 5), leave all parameters as they are set by default and click **Next**. Note that the default settings are set for somatic variant detection, and for different applications (such as germline variant detection), the frequency cut-off and the would typically need to be increased.

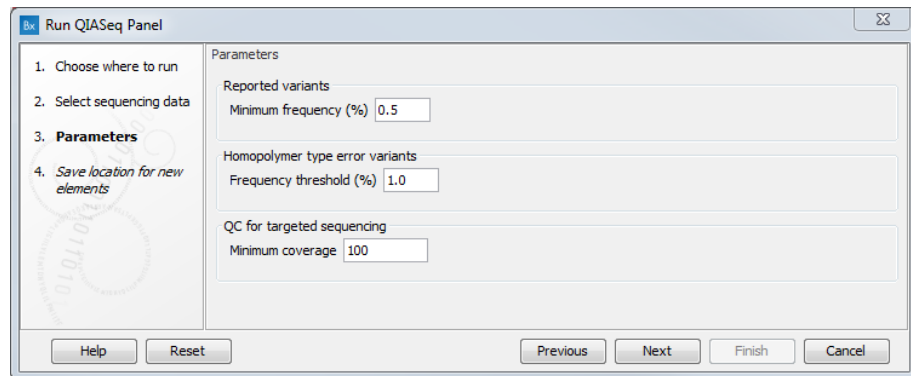




Figure 5: Leave all parameters as set by default.

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 6):

- a Genome Browser View 

- a UMI Groups Report 
- a read mapping of the UMI Reads 
- a coverage report and a coverage graph
- two variant tracks: the Unfiltered Variants is output before the filtering steps, the Variants passing filters is the one used in the Genome Browser View.

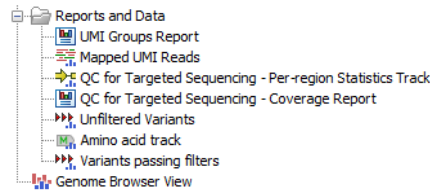


Figure 6: Output from the BRCA1 and BRCA2 Panel Analysis workflow.

Double click on the Genome Browser View. The file opens in a split screen with the Browser View 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**".

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") as low as 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 (figure 7). This allows you to view the coverage and the context of this variant, e.g., Mapped UMI Reads, Amino acid track, and so on.

### Change the post filtering steps on a workflow

You may be interested in changing some of the parameters set by default for the BRCA1 and BRCA2 Panel Analysis workflow, for example if you think the filtering is too stringent.

1. Open the Analyze QIAseq Panels guide and click on **Add custom panel**.
2. The application Targeted DNA is pre-selected, which allows you to choose the BRCA1 and BRCA2 Panel (DHS-102Z) panel from the drop-down menu list. The dialog is then filled up as seen on figure 8
3. Edit the name of the panel to your custom name.
4. Click on Additional parameters figure 9 and edit the filters for small (1-2 bp), medium (3-8 bp) and large variants (9 bp or longer).
5. You can now Run the new custom workflow. The Unfiltered Variants track will be unchanged, but you should get a different Variants passing filters track depending on how you changed the settings of the filters.

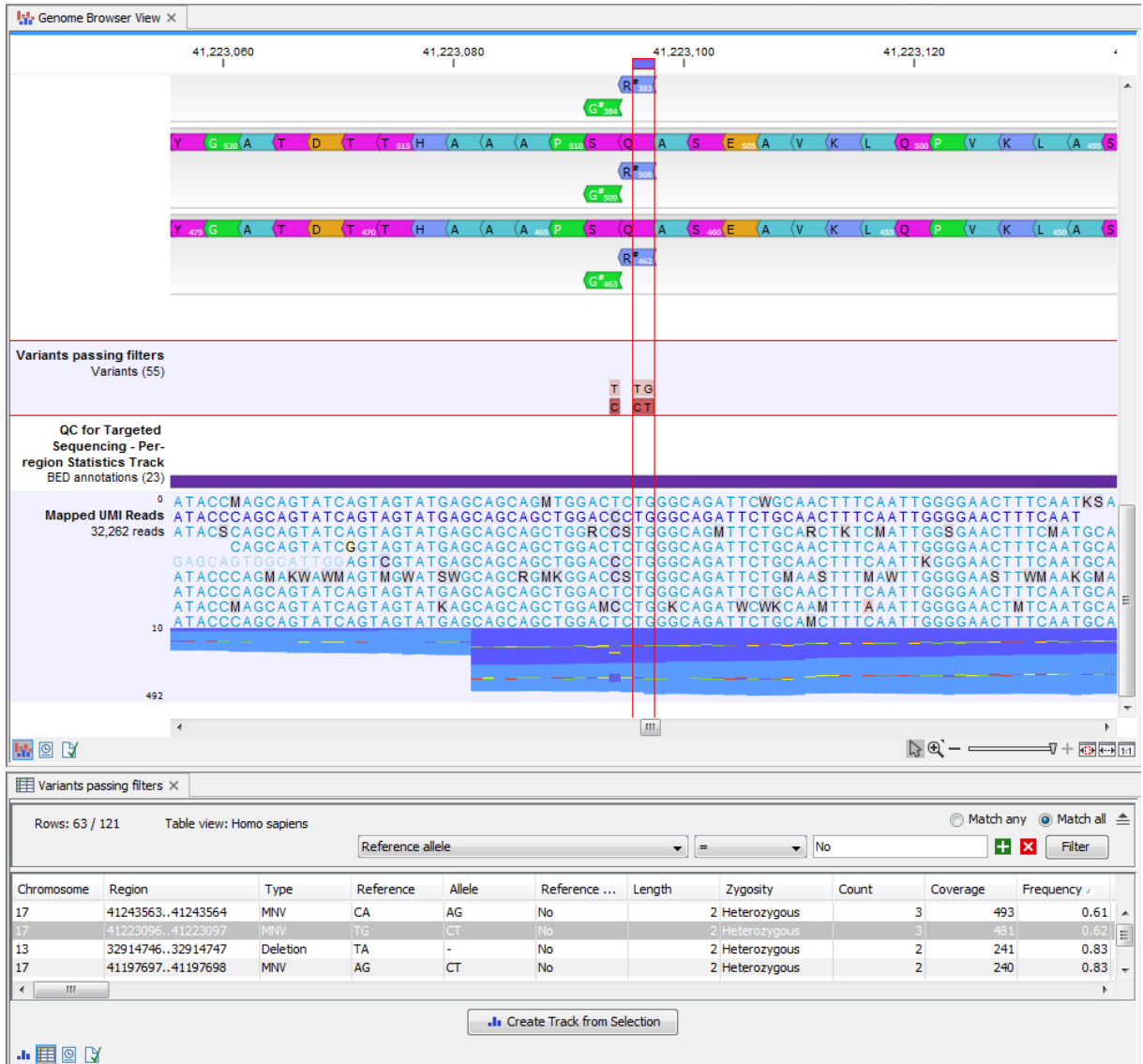


Figure 7: Genome Browser View.

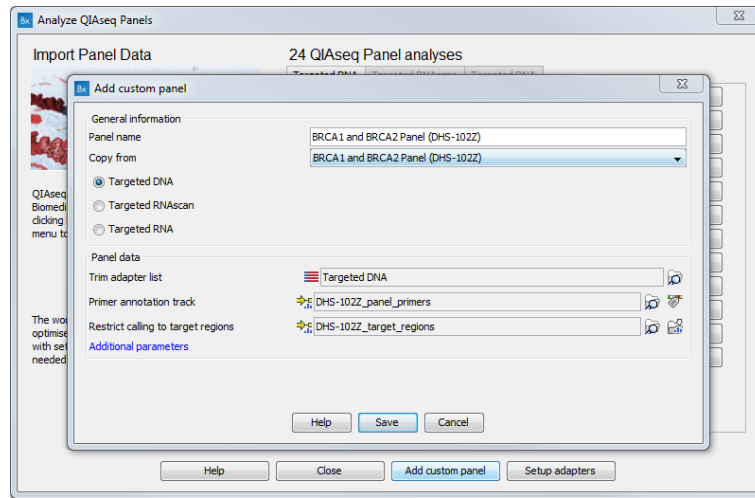


Figure 8: Create a custom workflow from an existin workflow template.

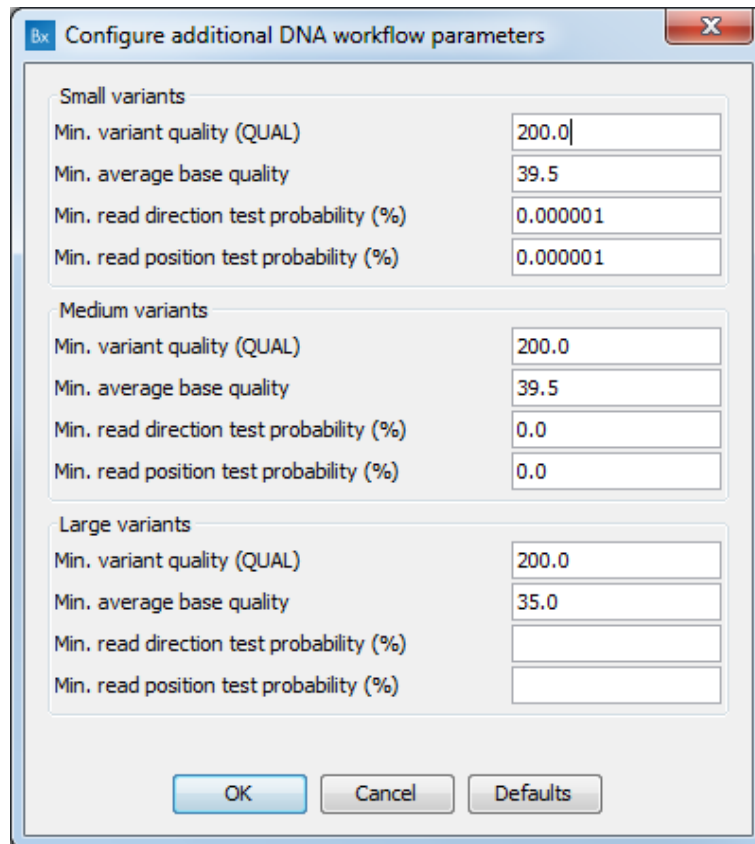


Figure 9: Post-filtering parameters can be edited in a custom workflow.