



# Tutorial

## Fusion Detection Using QIAseq RNAscan Panels

September 22, 2022

— Sample to Insight —

## Fusion Detection Using QIAseq RNAscan Panels

This tutorial uses the capabilities of CLC Genomics Workbench and the Biomedical Genomics Analysis plugin to detect fusion events in sequences generated using a QIAseq Targeted RNAscan Panel. The plugin includes an Analyze QIAseq Panels guide that facilitates the configuration and the running of QIAseq panels secondary analysis workflows. However, because we are working with a smaller data set here (limited to chromosome 10), we choose to show you in this tutorial how to proceed without using the guide, but using the Detect QIAseq RNAscans Fusions workflow instead.


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

- Import Illumina paired reads in the workbench.
- Generate a trim adapter list to remove the sequencing primers off the reads.
- Find fusion events with the **Detect QIAseq RNAscan Fusions** template workflow.

This tutorial makes use of a subset (reads mapping only to chromosome 10) of the sequencing reads obtained using a QIAseq Targeted RNAscan Panel.





**Prerequisites** For this tutorial, you must be working with the CLC Genomics Workbench with Biomedical Genomics Analysis plugin 20.0 or higher installed. How to install plugins is described in the [manual](#).

**Download and import data** Go through the following steps to download and import the data into the Workbench.

1. Download the [tutorial data from our website](#) and unzip it in the location of your choice on your computer.
2. Start the workbench.
3. Import the reads via the toolbar: **Import**  | **Illumina**
  - Select the two fastq paired files.
  - Under "General options" section, ensure the **Paired reads** and **Discard read names** checkboxes are checked.
  - In the "Paired read orientation" section, ensure the **Paired-end (forward-reverse)** option is checked.
  - Set the **Minimum distance** to 1 and the **Maximum distance** to 1000 (default values).
  - In the "Illumina options" section, ensure the **Remove failed reads** option is checked.
  - Click **Next**.
4. Click **Save** in the wizard page that appears, choose the folder you wish to save the reads to (you can create a new folder dedicated to this tutorial for example) and click **Finish**.

## Run Detect QIAseq RNAscan Fusions

The Detect QIAseq RNAscan Fusions template workflow can be found in the Toolbox here:

**Template Workflows** | **Biomedical Workflows**  | **QIAseq Panel Analysis**  | **QIAseq Analysis Workflows**  | **Detect QIAseq RNAscan Fusions** 

1. Double-click on the Detect QIAseq RNAscan Fusions template workflow to run the analysis. If you are connected to a CLC Server via your Workbench, you will be asked where you would like to run the analysis. We recommend that you run the analysis on a CLC Server when possible.
2. Select the sequencing reads that should be analyzed (figure 1).

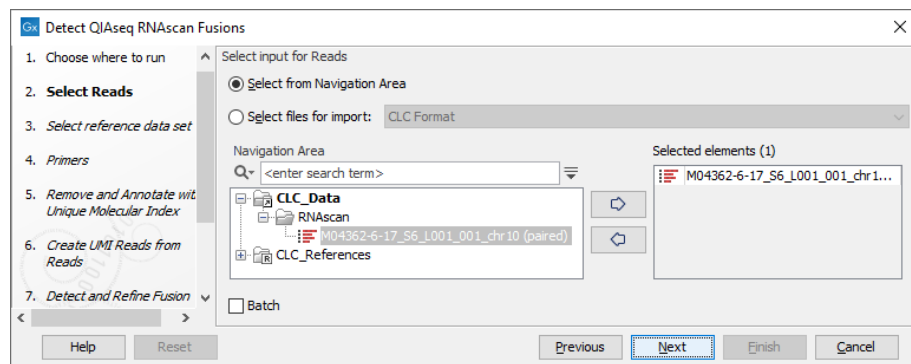


Figure 1: Select the sequencing reads by double-clicking on the file name or by clicking once on the file name and then on the arrow pointing to the right hand side.

3. Select the fusion Detection Using QIAseq RNAscan Panels data set situated in the QIAGEN Tutorial section (figure 2). This tutorial data set is designed to only contain the chromosome 10 for each reference. Download the reference data set to your computer. Selecting the full data set from the QIAGEN Active section will increase the running time of this tutorial.
4. The primer track covering only chromosome 10 is preselected since this track is bundled with the tutorial reference data set (figure 3). If you are using the full data set you will be asked to select the relevant primer track.
5. In the Remove and Annotate with Unique Molecular Index dialog the "Read structure" should be set to "Paired end reads (Index on Read 2)" (figure 4).
6. In the Create UMI reads from Reads dialog the "Read structure" should be set to "Paired end reads"
7. In the Detect and Refine Fusion Genes dialog, check the option "Detect exon skipplings" (figure 5) and click **Next**.
8. 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**.

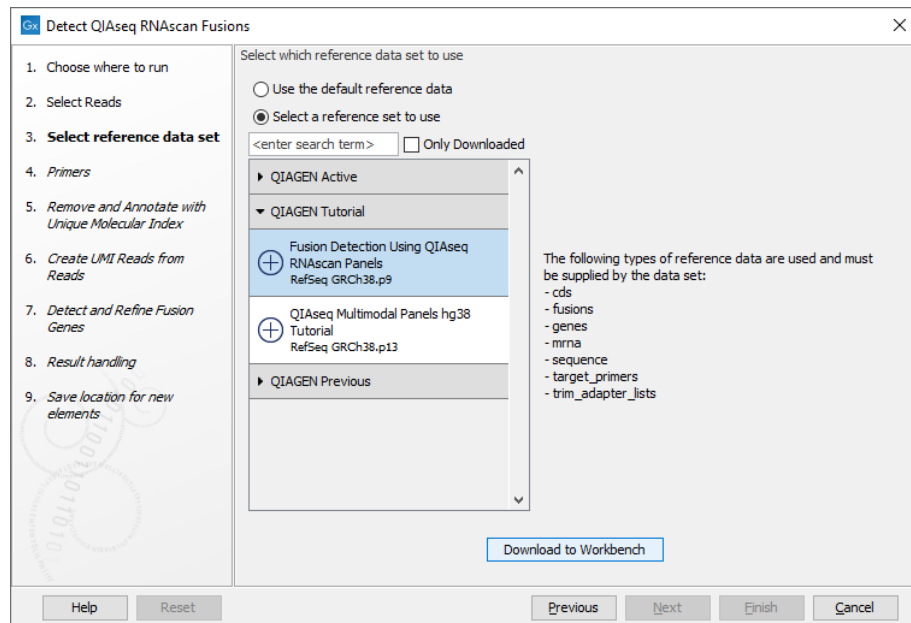


Figure 2: Select the relevant QIAGEN reference data set.

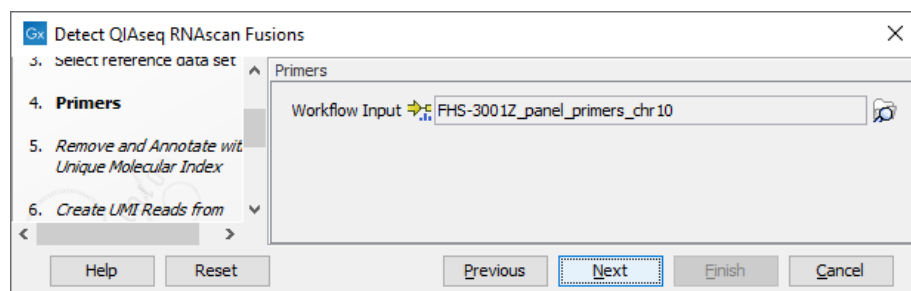


Figure 3: The primer track in the tutorial reference data set is preselected

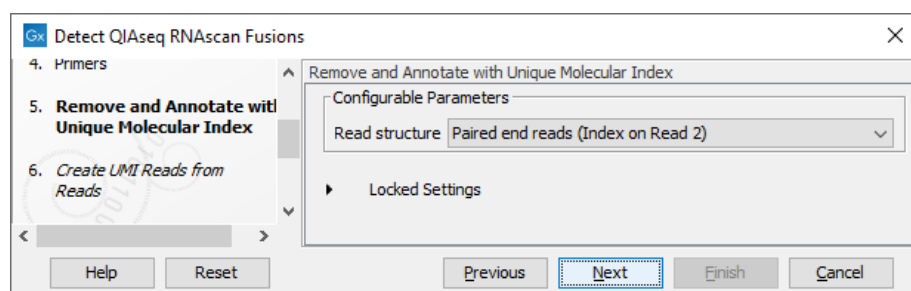


Figure 4: Specify the UMI read structure

### Output from the Detect QIaseq RNAscan Fusions workflow

The Detect QIaseq RNAscan Fusions workflow produces a series of output. Before checking the detected fusions, users are usually recommended to review the QC for RNAscan Panels Report, and in particular, to verify that no DNA contamination was found in the sample. Also the reference genes included in the RNAscan panels should have been detected and their expression to that of the target genes should be reviewed (Target gene vs reference gene coverage ratio [%]). In the case of this tutorial, the data was limited to chromosome 10 only and the QC for RNAscan

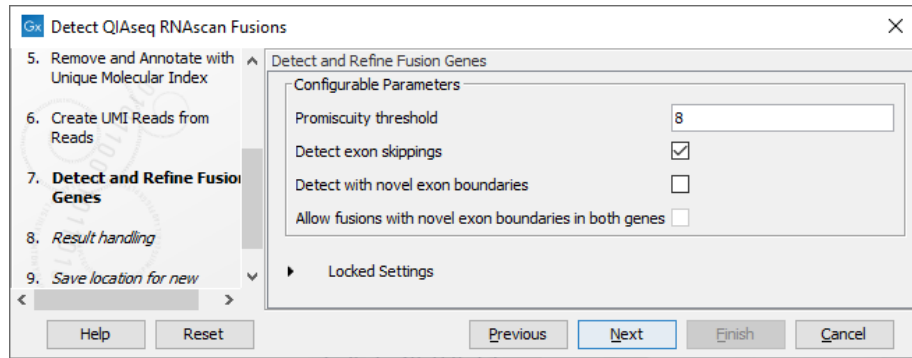


Figure 5: Choose to detect exon skipplings.

Panels Report is therefore incomplete.

The easiest way to review the results is to open the Genome Browser View (Fusions) (figure 6).

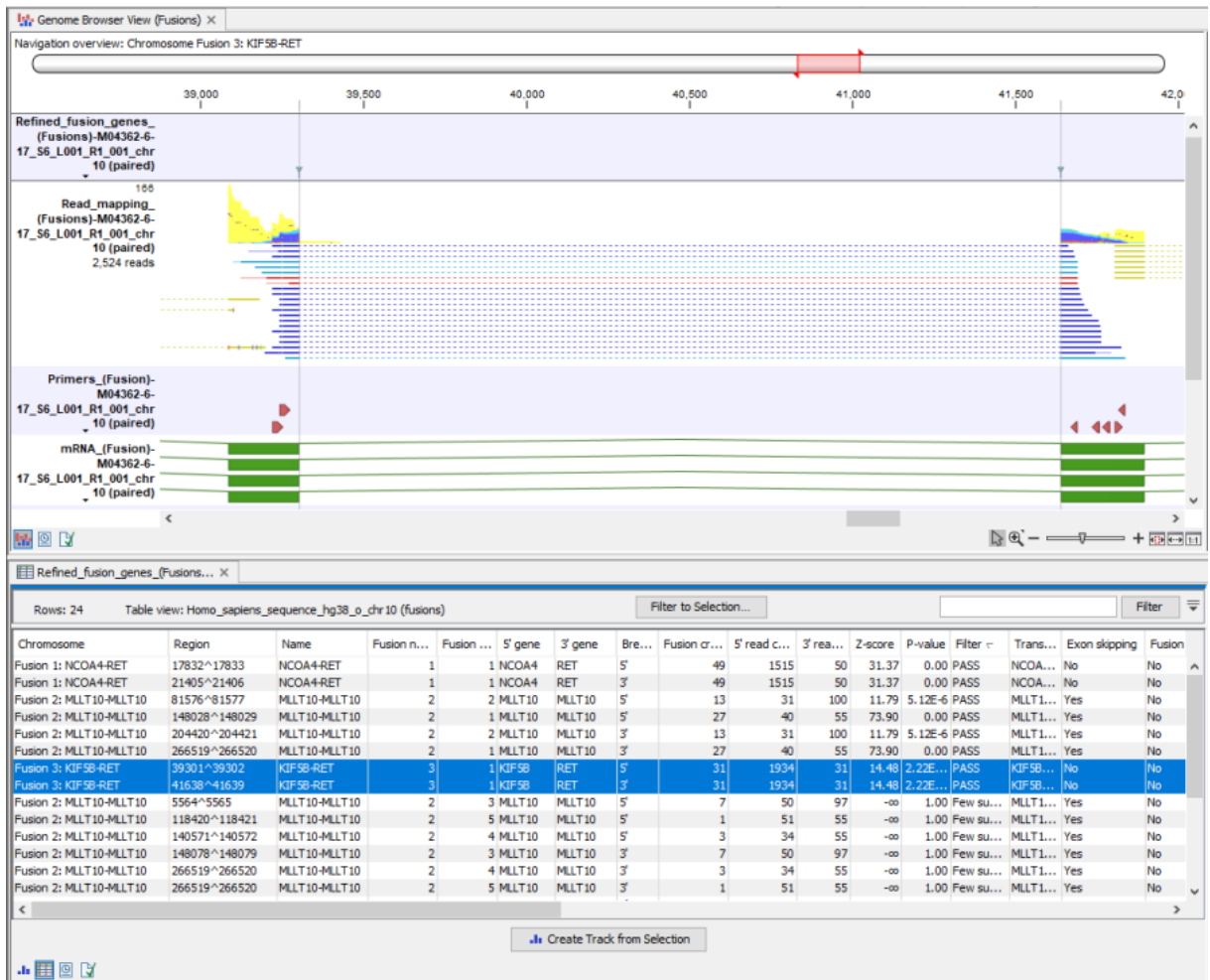


Figure 6: A Genome Browser View (Fusions) showing the KIF5B-RET fusion.

Double-click on the Fusion genes (fusions) track name. The fusion track will open as a table in split view, below the Genome Browser View. You can sort and filter the table using one of the

metrics: Fusions that get the **Filter** annotation **PASS** have not been caught by any filter and are therefore the most trustworthy fusions. Also, sorting the table using the fusion **Number** column will move the most trustworthy fusions to the top of the table. Note that one of the three fusions with a "PASS" is an exon skipping fusion, i.e., a same-gene fusion event where the 5' breakpoint is upstream of the 3' breakpoint. The 5' breakpoint is shown in figure 7.

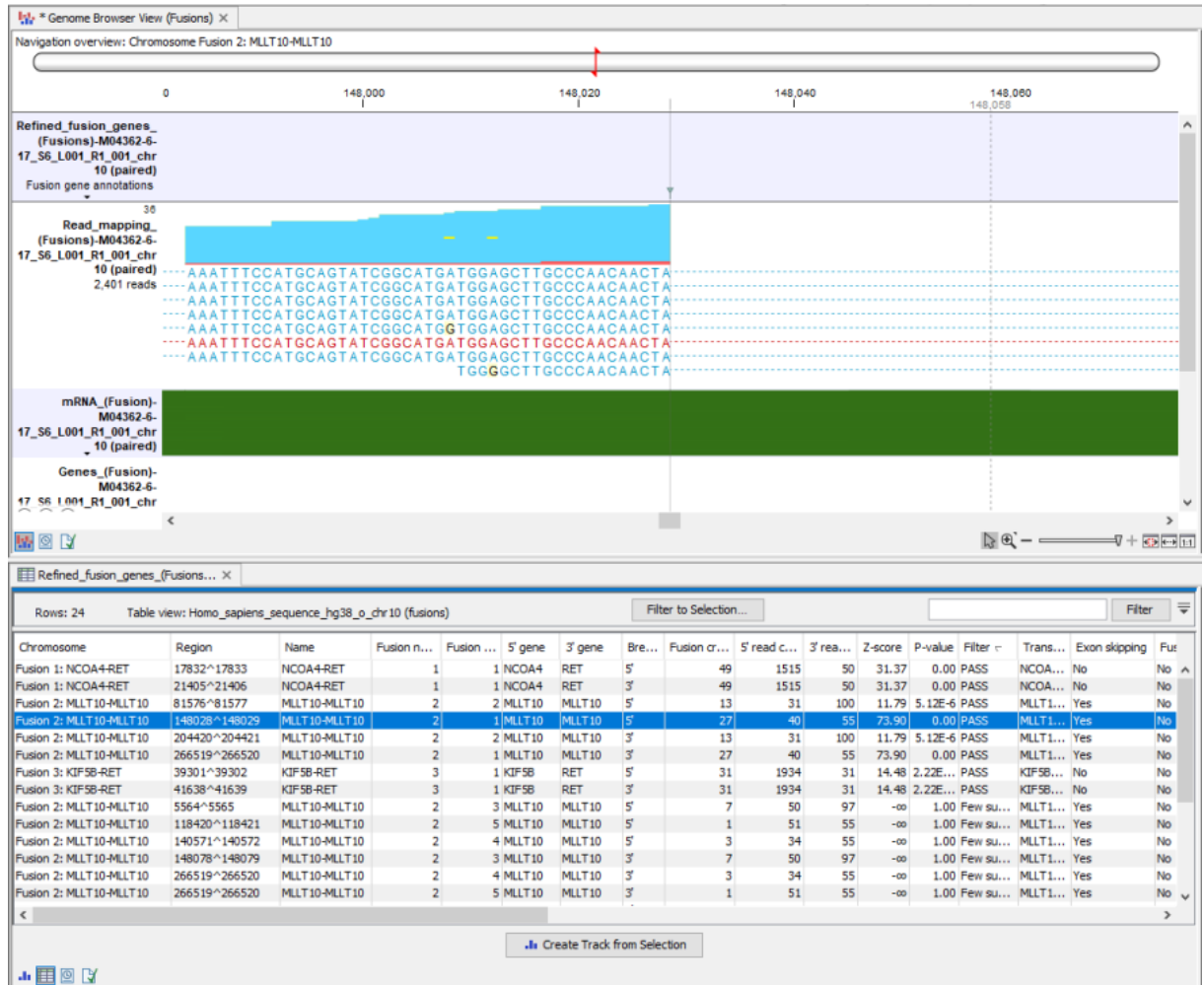


Figure 7: A Genome Browser View (Fusions) showing the MLLT10-MLLT10 exon skipping fusion.

- Click on a fusion event in the table to zoom in to its location in the read mapping. The reads supporting the detected fusion can now be reviewed. Each fusion breakpoint is represented by a row in the table. Two breakpoints with the same Fusion number and same Fusion pair represent one fusion event. A fusion must be supported by reads with spliced alignments, where the splice donor site is at the 5' breakpoint of the fusion and the splice acceptor site is at the 3' breakpoint of the fusion. In the mapping track, reads with spliced alignments are those with sections that map before the 5' breakpoint and after the 3' breakpoint, with splicing indicated between these regions by dotted lines. Note that the fusion chromosome contains intronic sequence between the two breakpoints and that there can be multiple breakpoints on each fusion chromosome.
- Open the Genome Browser View (WT) and locate the read mapping from the first iteration

in the Navigation Area where you saved the results in a subfolder named "Tracks". Then click on the track, hold down the mouse button and drag the read mapping from the first iteration from the Navigation Area into the Genome Browser View (WT). A blue line indicate where the track will show up in the Genome Browser View. See figure 8.

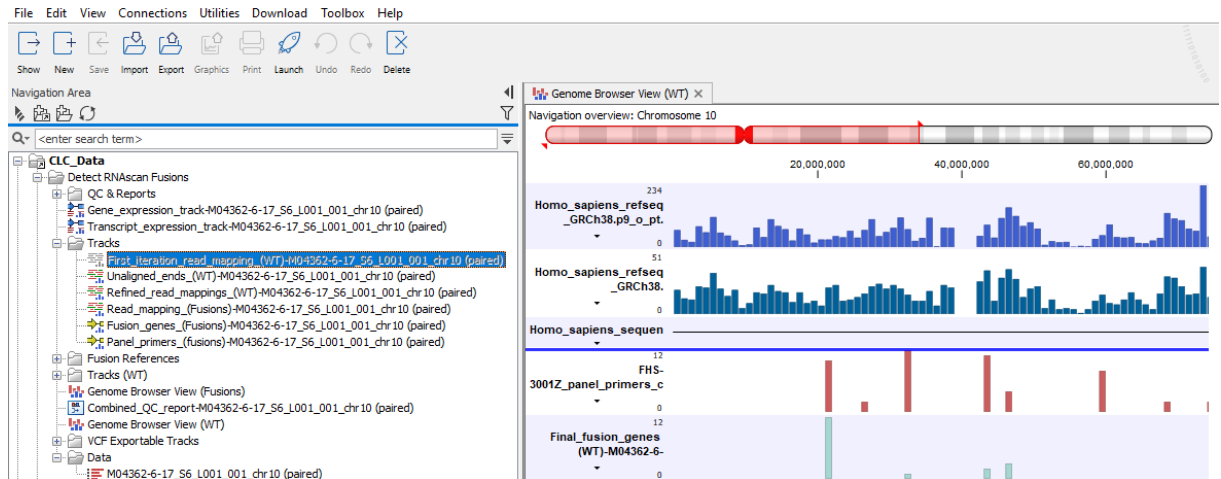


Figure 8: Dragging read mappings (first iteration) into the Genome Browser View (WT).

- Investigate how well the reads maps to the WT genes at the breakpoints by looking at the number of fusion crossing and fusion spanning reads, as well as the estimated Z-scores and p-values in the different Fusion genes tracks.

In cases where, other than expected, no reads map back to fusion genes in the fusion track, this eyeballing approach allows for lightweight troubleshooting.

Note that the read mapping track displays the mapping of reads to the transcript sequences. Reads in paired reads are in blue, and single reads, including those from broken pairs, are displayed in green (forward) and red (reverse). Further explanation of the coloring of reads in mappings is described in the [manual](#).