Tutorial

Fusion Detection Using QIAseq RNAscan Panels

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Sample to Insight

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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** ready-to-use 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 installed. How to install plugins is described here: http://resources.qiagenbioinformatics.com/manuals/clcgenomicsworkbench/current/index.php?manual=Install.html.

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

1. Download the sample data from our website: http://resources.qiagenbioinformatics.com/testdata/Fusion_Gene_Detection_tutorial.zip 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).
    - Click **Next**.
4. Click on the button labeled **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 ready-to-use workflow can be found in the Toolbox here:

Ready-to-Use Workflows | QIAseq Panel Analysis | QIAseq Analysis Workflows | Detect QIAseq RNAscan Fusions

1. Double-click on the Detect QIAseq RNAscan Fusions ready-to-use 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).

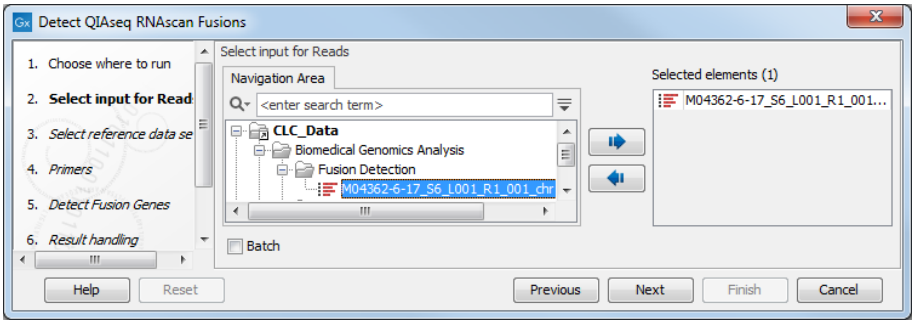


Figure 1: *Select the sequencing reads by double-clicking on the file name or by clicking once on the file name and then on the arrow pointing to the right hand side.*

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. Selecting the full data set from the QIAGEN Active section will increase the running time of this tutorial.

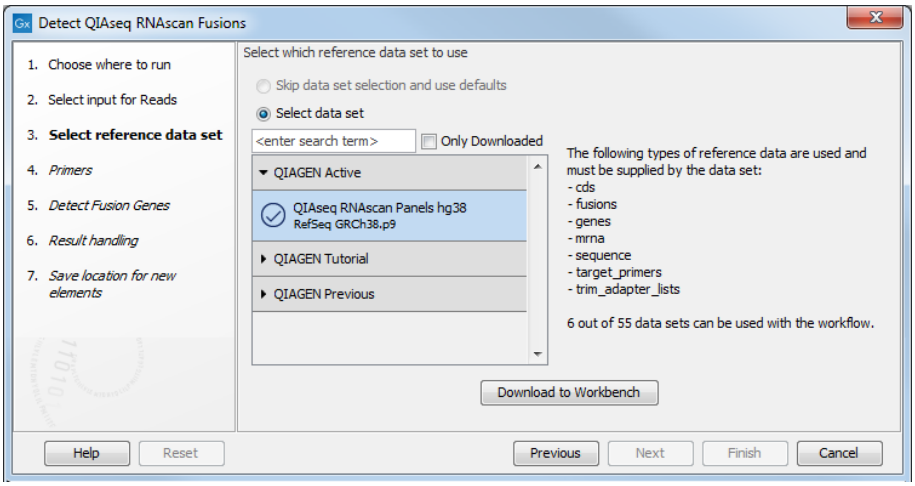


Figure 2: *Select the relevant QIAGEN reference data set.*

4. Select the **primers track** available in the CLC_References folder once the tutorial Reference Data Set has been downloaded (figure 3).

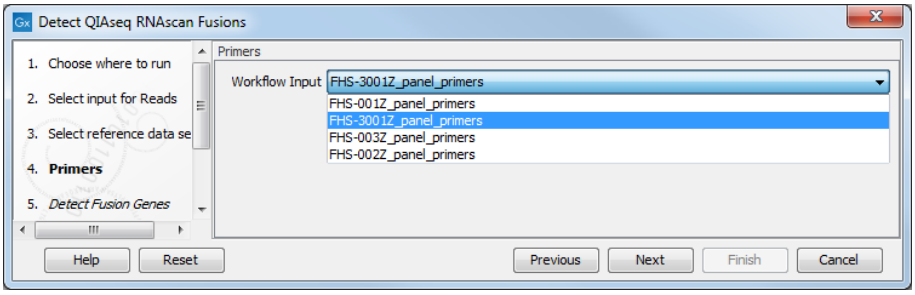


Figure 3: *Select the relevant primer track.*

5. In the Detect Fusion Genes dialog, check the option "Detect exon skippings" (figure 4) and click **Next**.

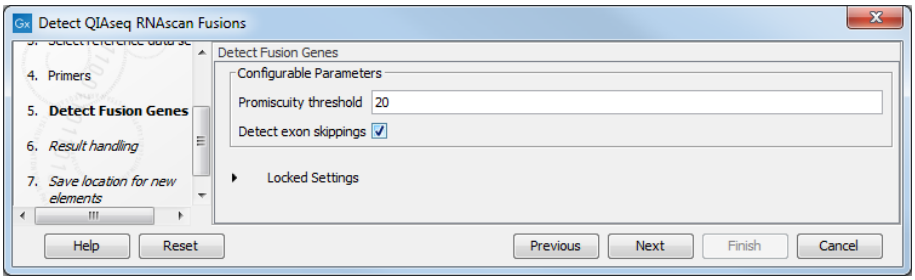


Figure 4: *Choose to detect exon skippings.*

6. 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 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 Panels Report is therefore incomplete.

The easiest way to review the results is to open the Genome Browser View (Fusions) (figure 5).

Double-click on the Fusion genes (fusions) track name (top left of the Genome Browser View). 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. This same fusion is found to be not in frame (information available because a CDS track was specified as input), as seen in figure 6.

Clicking on a fusion event in the table will zoom in to its location in the UMI read mapping,

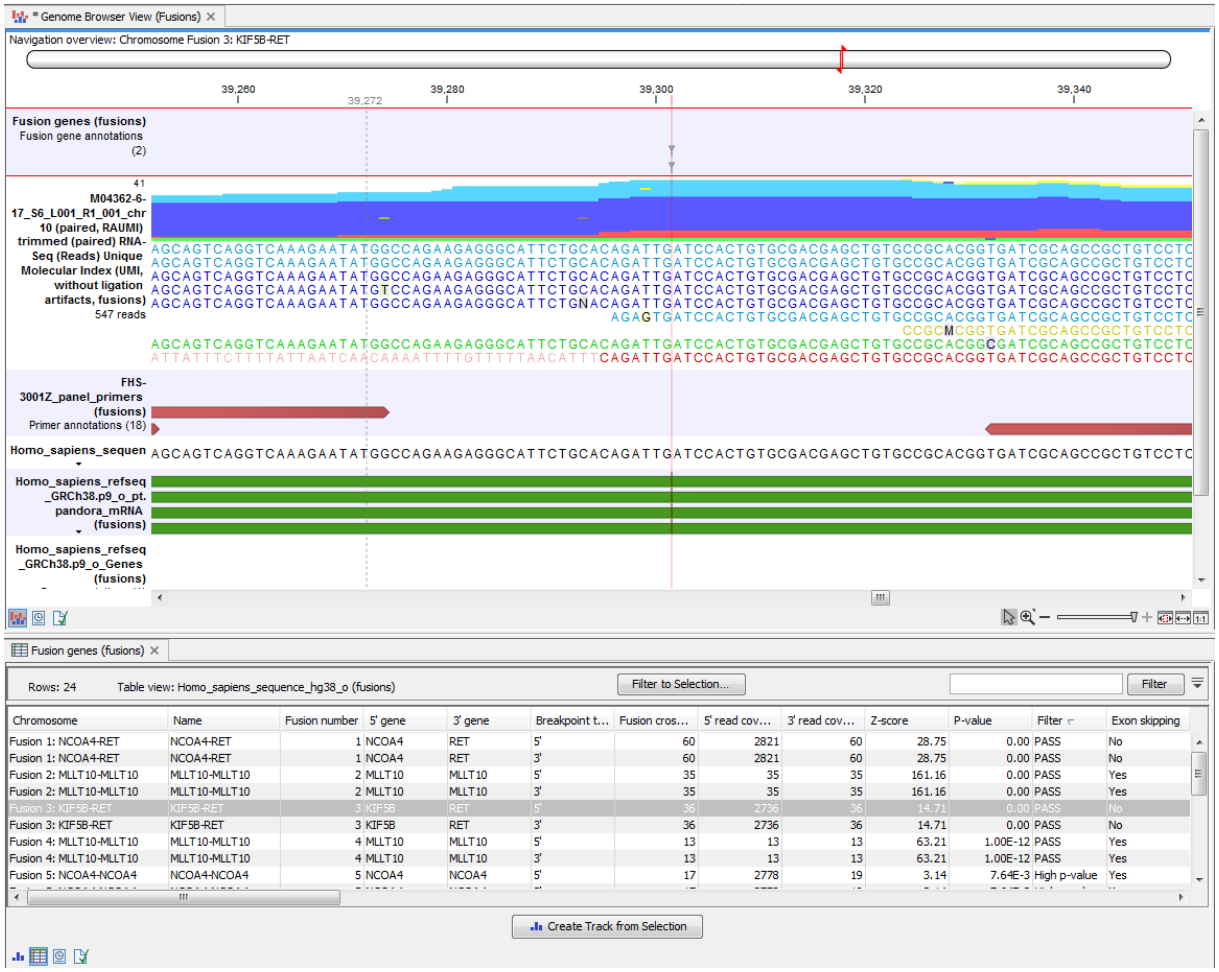
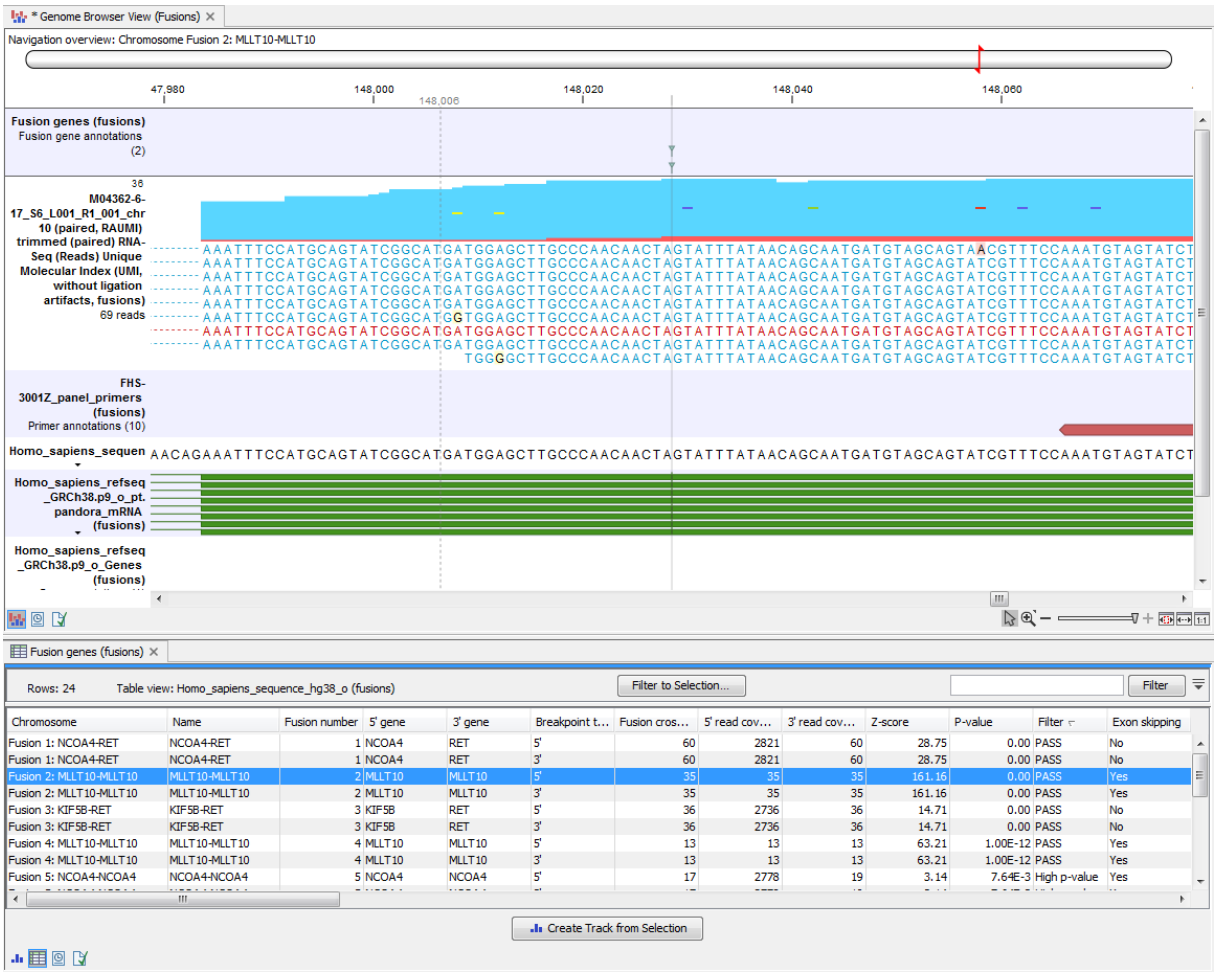


Figure 5: *A Genome Browser View (Fusions) showing the KIF5B-RET fusion.*

allowing you to review the UMI reads supporting the detected fusion. Note that each fusion event is represented by two rows in the table, allowing you to visualize successively both breakpoints of each fusion. You can estimate the evidence for the fusion by studying the Mapped UMI Reads that aligns to one side of a fusion, and the Mapped Unaligned Ends that, after re-mapping, align to the other side of the fusion. It is also possible to assess in the Genome Browser View (WT) know how well the reads maps to the WT genes at the breakpoints. Besides, the WT tracks that are not included in the Genome Browser View (read mappings of the first iteration, and the breakpoint track from the detect tool) can be dragged from the Navigation Area into the Genome Browser View in order to see the results from the Detect Fusion Genes tool. For example, look 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. This could be interesting in cases where no reads map to the fusion genes in the fusion tracks.

Note that the Mapped UMI track displays the mapping of UMI reads to the transcript sequences. Paired reads still in proper pairs are blue. Forward reads are green, reverse reads are red. Unaligned ends of mapped reads are shown in a lighter shade of the respective read color. Multi-match reads, i.e., reads that could have been mapped to another position within the mapping criteria used, are in yellow.



Chromosome	Name	Fusion number	5' gene	3' gene	Breakpoint t...	Fusion cros...	5' read cov...	3' read cov...	Z-score	P-value	Filter	Exon skipping
Fusion 1: NCOA4-RET	NCOA4-RET	1	NCOA4	RET	5'	60	2821	60	28.75	0.00	PASS	No
Fusion 1: NCOA4-RET	NCOA4-RET	1	NCOA4	RET	3'	60	2821	60	28.75	0.00	PASS	No
Fusion 2: MLLT10-MLLT10	MLLT10-MLLT10	2	MLLT10	MLLT10	5'	35	35	35	161.16	0.00	PASS	Yes
Fusion 2: MLLT10-MLLT10	MLLT10-MLLT10	2	MLLT10	MLLT10	3'	35	35	35	161.16	0.00	PASS	Yes
Fusion 3: KIF5B-RET	KIF5B-RET	3	KIF5B	RET	5'	36	2736	36	14.71	0.00	PASS	No
Fusion 3: KIF5B-RET	KIF5B-RET	3	KIF5B	RET	3'	36	2736	36	14.71	0.00	PASS	No
Fusion 4: MLLT10-MLLT10	MLLT10-MLLT10	4	MLLT10	MLLT10	5'	13	13	13	63.21	1.00E-12	PASS	Yes
Fusion 4: MLLT10-MLLT10	MLLT10-MLLT10	4	MLLT10	MLLT10	3'	13	13	13	63.21	1.00E-12	PASS	Yes
Fusion 5: NCOA4-NCOA4	NCOA4-NCOA4	5	NCOA4	NCOA4	5'	17	2778	19	3.14	7.64E-3	High p-value	Yes

Figure 6: *A Genome Browser View (Fusions) showing the MLLT10-MLLT10 exon skiping fusion.*