QIAGEN

Tutorial

Compare TMB Scores and MSI Statuses from QIAseq Tumor Mutational Burden Panels

May 10, 2019

Sample to Insight

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Compare TMB Scores and MSI Statuses from QIAseq Tumor Mutational Burden Panels

This tutorial uses the capabilities of CLC Genomics Workbench with the Biomedical Genomics Analysis plugin to calculate reliable and consistent TMB score and MSI status values.

QIAGEN offers the QIAseq Tumor Burden Mutation panel assay (DHS-8800Z) covering a significantly larger region of the genome than other Targeted DNA panels and including gene families, which increases the difficulty of variant calling especially with regards to specificity. Through a series of tools and filters, the Identify TMB Status ready-to-use workflow has the ability to accurately call variants and to compute a TMB score (total number of somatic mutations in a defined region of a tumor genome) that can be used for classification as TMB low, intermediate or high. High TMB scores have been shown to be correlated with improved patient response rates to immune check-point inhibitors [A. Stenzinger et al., 2019].

In addition, the DHS-8800Z QIAseq Targeted DNA panel covers loci useful for Microatellite Instability Status inference. This status measures the statistical variation of the length distribution of several microsatellite loci, and compares the statistical variation of the test sample with a normal samples' baseline (included in QIAGEN Reference Data Set available for download in the Workbench). If the proportion of unstable microsatellite loci is higher than the predefined threshold, then the sample is considered unstable. Many patients with MSI-High tumors have had a positive response to immunotherapy treatments. In addition, The US Food and Drug Administration (FDA) has granted accelerated approval to pembrolizumab for pediatric and adult patients with microsatellite instability (MSI)-High, leading to the first approved treatment for any solid tumor with this biomarker irrespective of the tumor's origin [Chang et al., 2018].

This tutorial does not include running the Identify TMB Status ready-to-use workflow because of the time constraint we have on tutorials. We have chosen to provide you with a subset of the results of this workflow performed on 5 samples to cover the following:

- Import filtered variant tracks and read mappings in the Workbench.
- Run the Calculate TMB Score tool to output TMB Score reports for each sample.
- Run the Detect QIAseq MSI Status (beta) workflow on these samples for comparison.

This tutorial should run within 20 min on a regular laptop. If you wish to run the Identify TMB Status ready-to-use workflow, the original reads can be found on our website: https://www.qiagenbioinformatics.com/support/example-data/

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/TMB_MSI_tutorial.zip` and save it on your computer. For each sample, we provide:
   - A **Read Mapping** of the UMI Reads ( )
   - A Variant track: The **Filtered variant** track ( ) includes all somatic variants that remained after the filtering performed in the Identify TMB Status Ready-to-Use workflow and that is input Calculate TMB Score tool.
2. Start the workbench.
3. Import the reads via the toolbar: **Import** ( ) | **Standard Import**.
4. Select the `TMB_MSI_tutorial.zip` file and leave the option to "Automatic import". Click **Next**.
5. Select the location in the Navigation Area of your Workbench where the data should be saved, and click **Finish**.

In addition, you will need to import the Reference Data Set needed to run the tool and workflow demoed in this tutorial.

1. Click on the button References in the top right corner of the Workbench.
2. In the QIAGEN Sets tab, under the Reference Data Sets section, choose the QIAseq TMB Panels hg38 and click on **Download** (figure 1).

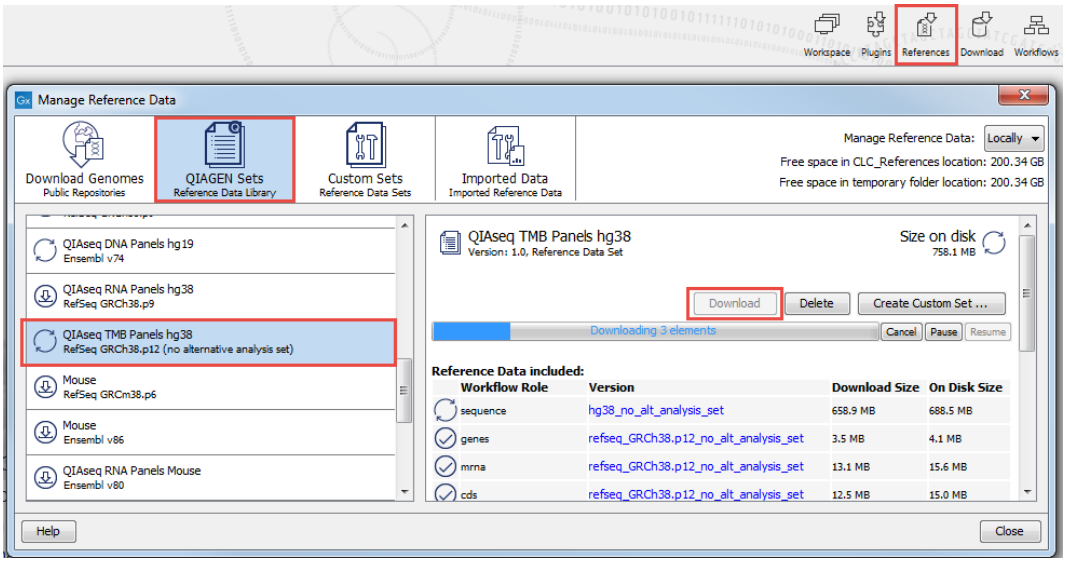

Figure 1: *Importing the relevant Reference Data Set.*

3. Once imported, the Reference data elements included in the set will be available in the CLC_References folder in the Navigation Area.

Run the Calculate TMB Score tool

1. Start the Calculate TMB Score tool from the Toolbox:

   Tools | **QIAseq Panel Expert Tools** | **QIAseq DNA Panel Expert Tools** ( ) | **Calculate TMB Score** ( )

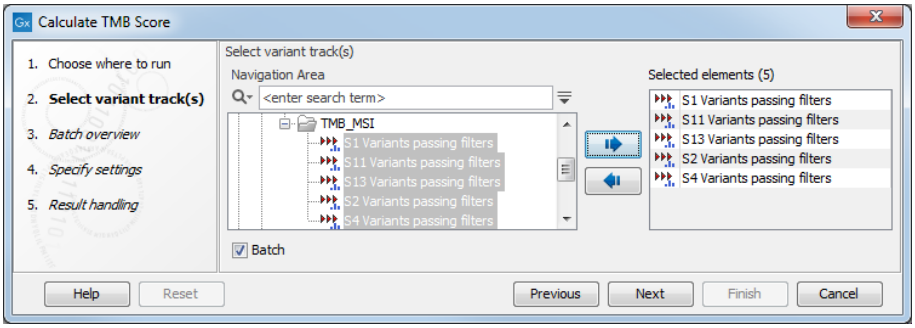

Figure 2: *Select the 5 variant tracks to generate the TMB Score reports in batch.*

2. The "Batch overview" dialog indicates the various batch units selected, and allow you to potentially choose which to include in the analysis. For this tutorial we have already selected all samples needed, so you can just click **Next**.

3. Three reference tracks specific to the DHS-8800Z panel need to be specified in order to calculate the TMB score. These files are all included in the CLC_References folder of the Navigation Area once you have download the Reference Data Set. You will find them in the QIAseq TMB Panels hg38 (RefSeq GRCh.38.p12 (no alternative analysis set)) folder, in three subfolders named **target_regions**, **masking_regions** and **dbsnp_tmb** (highlighted in blue in figure 3). Make sure you select the "Reference Data" tab (highlighted in red) to be able to locate easily the reference tracks that belong to a given Reference Data Set. The same tracks are also present in the "Navigation area" tab but organized differently.

   We leave all other settings at their default values as these have been configured to optimize sensitivity and specificity in selecting variants included in the TMB score calculation.

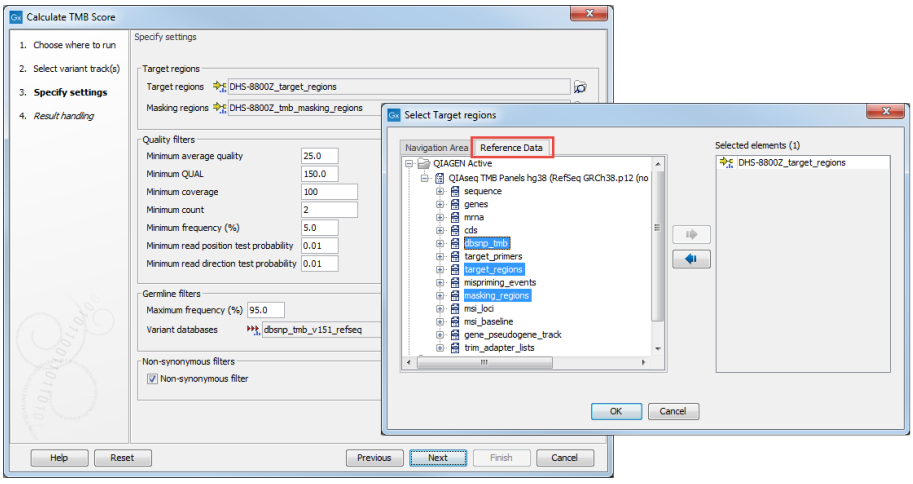

Figure 3: *Select the 5 variant tracks to generate the TMB Score reports in batch.*

4. In the next dialog, choose the location where you want your reports to be saved and click **Run**.

The tool will output two files for each sample: the **Variants passing filters (TMB Somatic)** track, containing the variants that were used to calculate the TMB score. Indeed, the tool applies successively another round of various quality, germline and non-synonymous filters before calculating the score.

The **TMB** report ( ) includes filtering statistics and the calculated TMB score and status. The TMB status is considered low if the TMB score is lower than 10; intermediate if the TMB score is between 10 and 15; and high if the TMB score is larger than 15. Note that these generic category cut-offs are only indicative and should not be used as cancer-specific classifications without proper validation by the user.

In addition to the TMB status, the report lists the length of the combined target regions, counts of various types of variants, and a value assigned to the tumor mutational burden calculated as the number of mutations per Mb. The quality filters statistics recapitulates how many variants were removed by the various filters applied by the tool. Finally, the report shows the frequency distributions of input and somatic variants.

Run the Analyze QIAseq Panel Guide

We will now run the TMB and MSI Panel (DHS-8800Z) workflow on all 5 samples with the Analyze QIAseq Panel guide.

1. Open the guide here:

   QIAseq Panel Analysis | **Analyze QIAseq Panel**

   and choose the TMB/MSI tab.

2. Click on the arrow to the right of the TMB and MSI Panel (DHS-8800Z) workflow, select the option **Somatic, Illumina (MSI)** as seen on figure 4 and click **Run**.

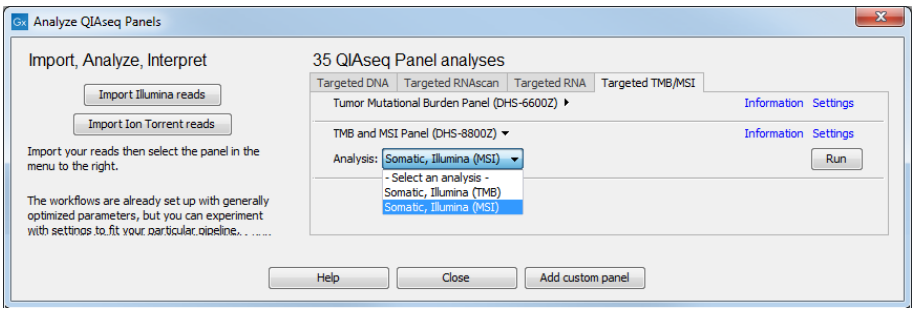

Figure 4: *Select the 8800Z panel and choose the MSI workflow.*

3. Check the batch mode (as highlighted in figure 5) before selecting the read mappings called **Mapped UMI Reads** that should be analyzed before clicking **Next**.

4. 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**.

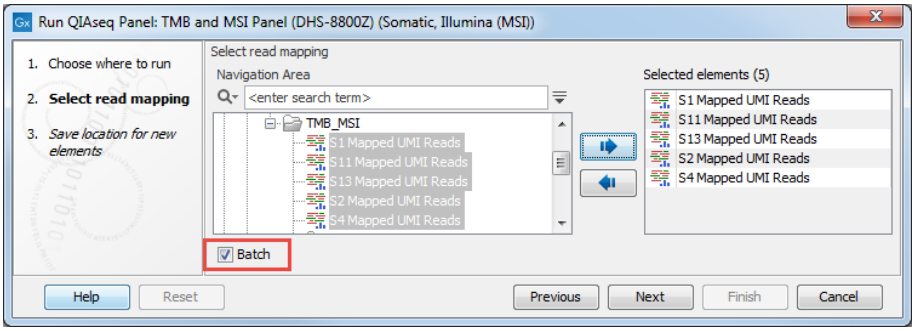

Figure 5: *Select the sequencing reads. Remember to run them in batch mode!*

The workflow will output 5 MSI reports. You can now open side by side for each sample the TMB and the MSI reports (as we did for S2 in figure 6). S2 is MSI low, and its TMB score is also low.

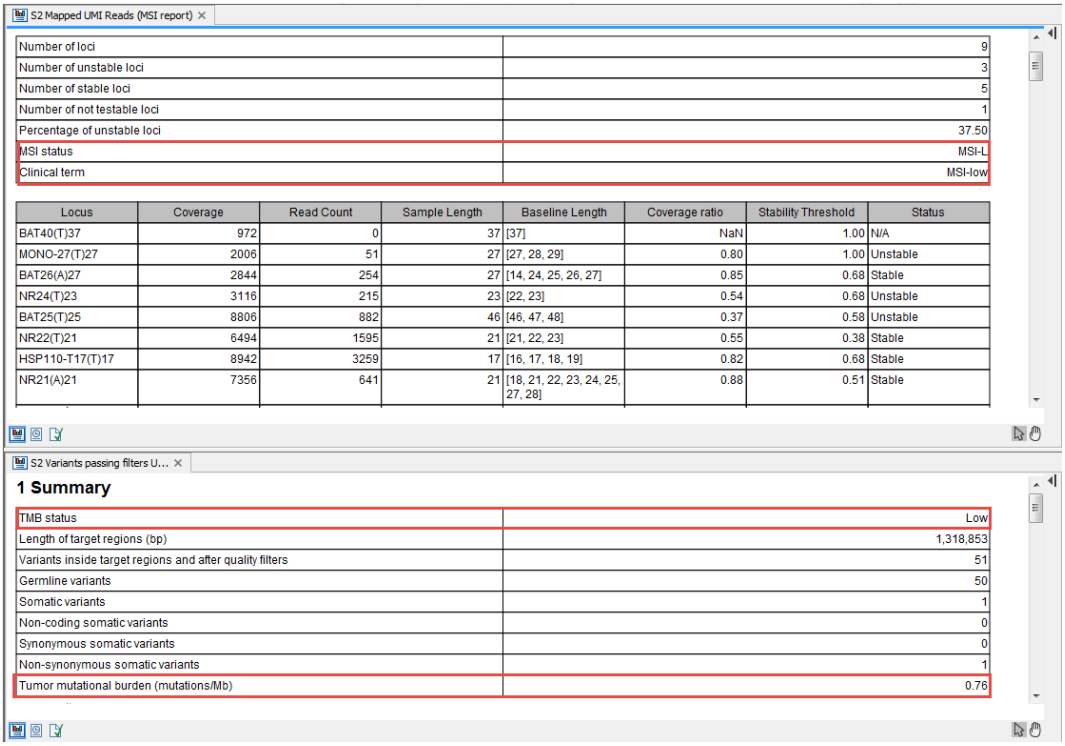

Figure 6: *TMB and MSI reports opened simultaneously.*

By opening the other samples in a similar way, you will notice that TMB and MSI are not always correlated: Samples 11 and 13 are both MSI-high, but one has a low TMB score (S13), while the other has a high TMB score (S11). The same situation is happening for the MSS samples S1 (low TMB score) and S4 (high TMB score). While the consequences of TMB score and MSI status on potential therapies are still under investigation, QIAGEN panels and bioinformatics solution for standardized and consistent assessment of both.

Bibliography

[A. Stenzinger et al., 2019] A. Stenzinger, J. A., Maas, J., Stewart, M., Merino, D., Wempe, M., and Dietel, M. (2019). Tumor mutational burden standardization initiatives:recommendations for consistent tumor mutational burdenassessment in clinical samples to guide immunotherapytreatment decisions. *Genes C*, pages 1–11.

[Chang et al., 2018] Chang, L., Chang, M., Chang, H., and Chang, F. (2018). Microsatellite instability: A predictive biomarker for cancer immunotherapy. *Applied I*, 26(2):e15–e21.