

# Ion Torrent™ Oncomine™ Myeloid Measurable Residual Disease (MRD) DNA Assay: Bioinformatics Methods and Outputs

The IQVIA Laboratories Ion Torrent™ Oncomine™ Myeloid Measurable Residual Disease (MRD) DNA Assay, developed by ThermoFisher, is a targeted NGS assay designed for the detection of Single Nucleotide Variants (SNVs) and insertions/deletions, including tandem duplications, in 33 myeloid disease-related genes. It leverages Ion AmpliSeq™ HD technology, including Unique Molecular Tags (UMT) for suppression of amplification artifacts. Paired with a bioinformatics assembly and variant calling pipeline, this assay allows for accurate and highly sensitive variant detection in whole blood or bone marrow samples.<sup>1</sup> The Oncomine™ Myeloid MRD analysis pipeline results in multiple data files and statistics. This document outlines these files and their contents.

## Methods

Sequencing data is analyzed on the Torrent Suite™.<sup>2</sup> Bases are called from raw sequencing data and reads are aligned using the Torrent Suite™ software to the Oncomine™ Myeloid MRD panel with hg19 as the human reference sequence. Binary Alignment Map (BAM) files are exported to Ion Reporter™.<sup>3</sup> On the Ion Reporter™ server, variants are called using the Torrent Variant Caller (TVC, v5.18)<sup>4</sup> and then annotated using the Oncomine™ Variant Annotator plugin (v3.2).<sup>3</sup>

## Standard deliverables

### Variant calls (VCF)

#### DEFAULT FILE NAMING CONVENTION:

[SAMPLE\_NAME]\*\_RAW.VCF

The raw VCF file contains the base calls at each position that is targeted by the Oncomine™ Myeloid MRD

panel, including homozygous reference calls, SNVs, and insertions/deletions, and is produced by the Ion Reporter™ using the Torrent Variant Caller.<sup>2</sup> One raw VCF file will be delivered for each sample.



#### **DEFAULT FILE NAMING CONVENTION:**

##### **[SAMPLE\_NAME]\*\_SMALL\_VARIANTS.VCF**

The small variants VCF file contains detected SNV and insertion/deletion variant calls targeted by the OncoPrint™ Myeloid MRD panel. This file is a subset of the raw VCF file containing only detected variants. One small variant VCF file will be delivered for each sample.

#### **DEFAULT FILE NAMING CONVENTION:**

##### **[SAMPLE\_NAME]\*\_TANDEM.VCF**

The tandem VCF file contains potential FLT3-ITD variant calls targeted by the OncoPrint™ Myeloid MRD panel. This file is a subset of the raw VCF file. One tandem VCF file will be delivered for each sample.

#### **BAM and BAI**

##### **DEFAULT FILE NAMING CONVENTION: [BARCODE]\_[SAMPLE\_NAME]\*.BAM AND [BARCODE]\_[SAMPLE\_NAME]\*.BAI**

The BAM file contains the aligned reads for each sample with the associated BAI file providing genomic indexing for the aligned reads. These files are produced by the Torrent Suite.<sup>2</sup> One set of BAM/BAI files will be delivered for each sample.

## Optional deliverables (additional fees may apply)

#### **FASTQ**

##### **DEFAULT FILE NAMING CONVENTION: [BARCODE]\_[SAMPLE\_NAME]\*.FASTQ.GZ**

Upon request, you may receive a FASTQ file containing the unaligned reads for each sample. One FASTQ file can be delivered for each sample.

## Appendix: Description of industry standard file types

#### **\*.bam and \*.bai**

BAM files are an industry-standard method for delivering alignments of one sequence to another in a compressed manner. This is a compressed file that contains the alignment of all sequence reads to the reference genome (the human genome, hg19). By themselves, BAM files cannot be used because they are compressed. However, when associated with a \*.bam.bai file (which is provided), they can be loaded into a genome viewer to visualize alignments. To visualize your alignments, you will need to download and install a genome browser. There are several available genome browsers, including Golden Helix's GenomeBrowse<sup>5</sup> and the Broad's Integrative Genomics Viewer.<sup>6,7</sup> In addition to viewing these files, you can convert these BAM files to SAM format files to review alignments line by line.

#### **\*.fastq or \*.fastq.gz**

FASTQ files are an industry-standard method for delivering unaligned read sequences and may be compressed (\*.fastq.gz). FASTQ files are text files, and they contain four lines for each entry. The first line is a sequence identifier, the second is the read sequence, the third is a plus sign (+) which serves as a separator, and the fourth is the base call quality scores in Phred encoding. For single-read sequencing, one FASTQ file is generated. For paired-end sequencing, two FASTQ files are generated. These large files can be viewed via the Unix/Linux command line but are more frequently used as input to downstream analysis software.



## \*.vcf

VCF files are an industry-standard method for delivering variations in genomic sequence. VCF files are tab-separated and can be opened in excel. Missing values are indicated by ".". VCF files contain a header consisting of lines that begin with "#". Lines in the header that begin with "##" denote keywords. Of note, the abbreviated terms in the FILTER, INFO, and FORMAT columns of the VCF are defined in the header. The last line of the header gives the column names for the body of the VCF. These columns are:

<b>CHROM</b>	The chromosome identifier from the reference genome.
<b>POS</b>	The position on the chromosome, with the first base being position 1.
<b>ID</b>	A list of unique identifiers, separated by ";". Common identifiers include those from dbSNP <sup>8</sup> , which begin with "rs" and those from COSMIC <sup>9</sup> , which begin with "COSM".
<b>REF</b>	The reference base(s). The location given in POS is the position of the first base of REF.
<b>ALT</b>	A list of alternate (non-reference) alleles, separated by ",".
<b>QUAL</b>	The quality score for the alternate allele indicated in ALT, with higher scores indicating more confidence in the call. Quality scores are provided in the phred scale.
<b>FILTER</b>	"PASS" indicates that the data at that position was sufficient to make a base call, whereas other values indicate a failure to pass a certain filter. Other values for FILTER are defined in the header with the keyword FILTER.
<b>INFO</b>	Additional information is provided as a short key with optional values, separated with ";". Each key is defined in the header with the keyword INFO and each item takes the format <KEY>[=<VALUE>[,<VALUE>]].
<b>FORMAT</b>	Keys for genotype information. Descriptions for each key are given in the header, with the keyword FORMAT.
<b>&lt;SAMPLENAME&gt;</b>	For the indicated sample, the values of each item listed in the FORMAT column. These values occur in the same order as given in FORMAT and are separated by ".". Multiple sample columns may be present.

## References

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