

# Oncomine immune repertoire assays for hemato-oncology research applications

Lymphoid cancers, including leukemias, lymphomas, or diseases such as multiple myeloma originate from the malignant transformation and clonal proliferation of one or more B or T cells. Every B and T cell expresses distinct receptors on its surface, which give rise to a vastly diverse immune repertoire. Using next-generation sequencing (NGS) technology, these unique receptor sequences can be used to assess clonality, detect rare clones, and measure somatic hypermutation (SHM).

NGS offers significant advantages over traditional approaches by providing sequence information, giving a more detailed view into repertoire (subclonal and intraclonal) diversity, offering ultrahigh sensitivity, and providing greater flexibility to multiplex.



### Clonality assessment

Confidently identify the dominant clone, measure clonal expansion, and determine its unique CDR3 sequence



### SHM analysis

Accurately quantify the frequency of SHM in the immunoglobulin heavy chain variable (IGHV) genes and determine the SHM status



### Rare clone detection

Detect rare B cell clones with high sensitivity and ultralow limit of detection (LOD) down to  $10^{-6}$ , and measure and compare the frequency of potential clones of interest

## Clonality testing and rare clone detection

NGS provides sequence-level resolution for clonality assessment, allowing you to detect expanded clones from polyclonal samples with very high specificity. Integrated bioinformatic tools let you easily assess clonal lineage and evaluate clonal evolution.

NGS offers a greater level of sensitivity when compared to traditional methods like flow cytometry. The ultralow LOD of  $10^{-6}$  (1 in 1,000,000 cells) enables you to detect extremely rare clones that traditional less-sensitive methods can miss.

Using proprietary Ion AmpliSeq™ technology, Ion Torrent™ Oncomine™ immune repertoire assays can target multiple immune receptor chains in a single reaction, which can

lead to increased rates of positive clonality detection (>90%). Clonality testing failures commonly result from somatic hypermutation preventing primer binding to the target sequence. The new Ion Torrent™ Oncomine™ pan-clonality assays can often overcome this challenge by including primers targeting multiple B or T cell receptor chains in a single reaction to increase the opportunity to detect a clone of interest from a sample.

### Ion Torrent™ Oncomine™ BCR Pan-Clonality Assay

This powerful and sensitive NGS assay can accurately assess clonality and detect rare clones in a range of sample types including blood, bone marrow, and formalin-fixed, paraffin-embedded (FFPE) tissues.

- Simultaneously sequence multiple receptor targets in a single reaction, including IGH, IgK, IgL rearrangements, as well as rearrangements containing C-intron (C-int) and kappa-deletion element (KDE)
- Enables reliable results with >90% positive clonality detection rates
- Confidently detect rare B cell clones with high sensitivity and ultralow LOD down to  $10^{-6}$
- Easily measure and compare the frequency of the potential clones of interest
- Enjoy simple and intuitive clonality assessment supported by the unique interactive visualizations and automated clonal lineage analysis features built into the Ion Reporter™ analysis software

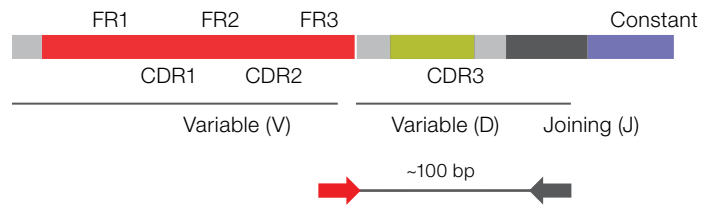
### Ion Torrent™ Oncomine™ BCR IGH SR Assay

- Assess clonality by targeting the CDR3 region of the IGH receptor
- Process DNA or RNA samples—use RNA input to detect rare B cell clones with high sensitivity (LOD:  $10^{-6}$ ) while maximizing cost efficiency
- Easily measure and compare the frequency of the potential clones of interest

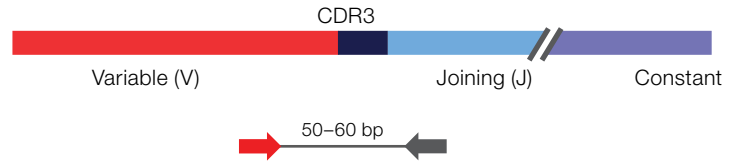
### Ion Torrent™ Oncomine™ BCR assays for secondary testing

For instances where secondary testing is required, additional panels are available with primer designs covering the framework 2 (FR2)-J regions and framework 3-distal FR3(d)-J regions. The FR3(d) primer set is a novel approach targeting a region of the FR3 region that tends to undergo a lower rate of somatic hypermutation, which increases the rate of primer binding.

#### A BCR heavy chain (IGH)



#### B BCR light chain (IgK/L)



#### C KDE/C-int

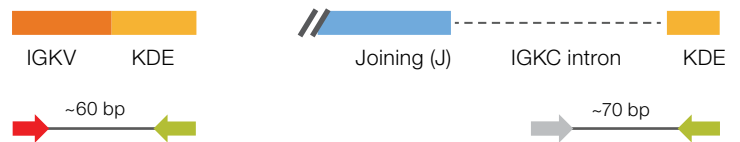


Figure 1. Oncomine BCR Pan-Clonality Assay primer design.

#### BCR IGH chain

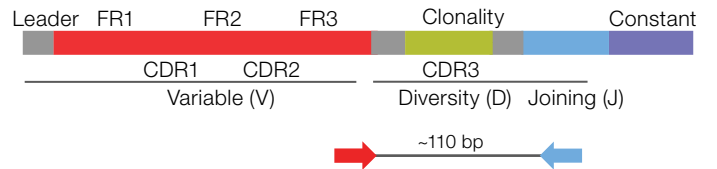


Figure 2. Oncomine BCR IGH SR Assay primer design.

#### BCR IGH chain

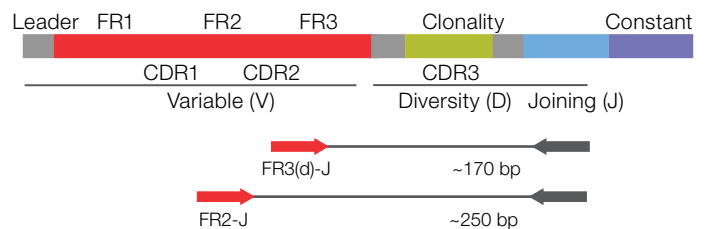


Figure 3. Primer design for the Oncomine BCR assays for secondary testing.

### Ion Torrent™ Oncomine™ TCR Pan-Clonality Assay

The Oncomine TCR Pan-Clonality Assay specifically interrogates the CDR3 region of the T cell receptor (TCR) beta and gamma chain genes.

- Sequence TCR beta and gamma targets in a single reaction
- Detect low-frequency T cell clones in peripheral blood, with sensitivity down to  $10^{-6}$
- Process samples with a low input requirement (50 ng DNA)
- Get results in a two-day turnaround time, complete with superior informatics for accurate clonality TCR beta and gamma chain sequence assessment without interference from primer bias

### TCRB/G

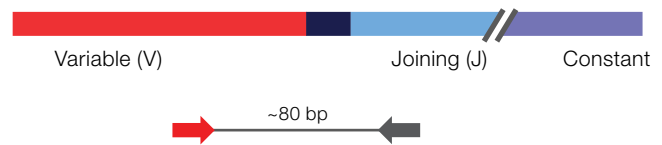


Figure 4. Oncomine TCR Pan-Clonality Assay primer design.

### Somatic hypermutation analysis

Following V(D)J recombination in developing lymphocytes, the IGHV gene undergoes somatic hypermutation. During this process, a series of point mutations are introduced to help confer greater repertoire diversity and enable higher affinity for potential antigens. The degree of somatic hypermutation is a key biomarker relevant for chronic

lymphocytic leukemia (CLL) research. Long-amplicon NGS assays provide highly accurate IGHV SHM quantification, while enabling efficient batch sample processing and simplifying the workflow when compared to traditional Sanger sequencing methods.

### Ion Torrent™ Oncomine™ IGHV Leader-J Assay

- Sequence from the leader to joining region of the BCR IGHV gene to assess SHM frequency
- The leader-J assay adheres to recommendations by the European Research Initiative on CLL (ERIC); these standards in CLL research aid in understanding of the biological relevance for immunogenetic analysis
- Accurately measure the level of SHM in the IGHV genes with the ultralow substitution error rate of the Ion Torrent™ platform
- Enjoy simple and intuitive analysis using the automated capability built into the bioinformatics software

### BCR IGH chain

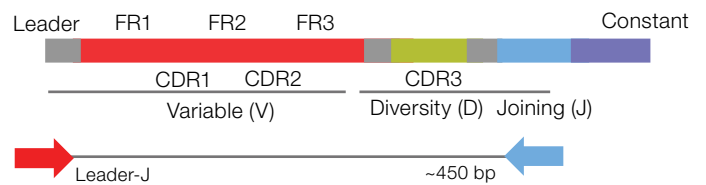


Figure 5. Oncomine IGHV Leader-J Assay primer design.

### Ion Torrent™ Oncomine™ BCR IGH-LR Assay

- Accurately measure the level of SHM from the FR1 region of the BCR IGHV gene
- Identify all isotypes (and subtypes) to expand immune repertoire research possibilities
- Process various sample types, including blood and bone marrow, using a low RNA input requirement (25 ng)

### BCR IGH chain

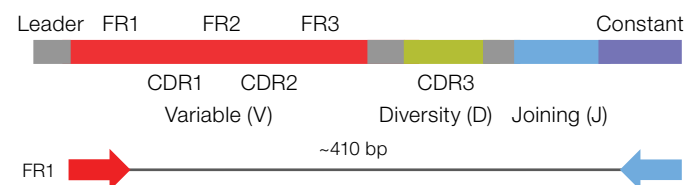


Figure 6. Oncomine BCR IGH-LR Assay primer design.

## Research assay summary table

Assay	Target(s)	Nucleic acid input	Sample types	Application(s)	Ion Torrent™ chip compatibility
Oncomine BCR Pan-Clonality Assay	BCR, IGH, IgK, IgL, KDE/ C <sub>int</sub> * (FR3-J)	gDNA	Whole blood, bone marrow, PBL,* PBMC,* sorted cells, fresh-frozen and FFPE-preserved tissue samples	Clonality, rare clone detection	Ion 530™, Ion 540™, and Ion 550™ Chips**
Oncomine BCR-SR Assay	BCR IGH (FR3-J)	gDNA, RNA			
Oncomine IGH FR3(d)-J Assay	BCR IGH (FR3(d)-J)	gDNA			
Oncomine IGH FR2-J Assay	BCR IGH (FR2-J)	gDNA			
Oncomine TCR Pan-Clonality Assay	TCRB, TCRG (FR3-J)	gDNA			
Oncomine IGHV Leader-J Assay	BCR IGH (Leader-J)	gDNA	Whole blood, bone marrow, PBL, PBMC, sorted cells	SHM	Ion 530 Chip
Oncomine IGH-LR Assay	BCR IGHV (FR1-C)	Non-FFPE RNA			
Oncomine IGH FR1-J Assay	BCR IGH (FR1-J)	Non-FFPE RNA			

\* PBL: peripheral blood leukocyte; PBMC: peripheral blood mononuclear cell.

\*\* The Ion 550 Chip is not compatible with the Oncomine IGH FR2-J Assay.

### Integrated workflow

As with all Oncomine assays, the complete workflow is fully integrated for speed and convenience. Start with any common sample type and easily prepare libraries for sequencing on the Ion GeneStudio™ S5 System.

Use either the Ion 530, Ion 540 or Ion 550 Chip to meet your throughput requirements. Integrated analysis software provides powerful visualization tools to simplify interpretation of results.



Sample types	Ion AmpliSeq™ library preparation	Ion Chef™ Instrument and Ion GeneStudio S5 system	Ion Reporter Software analysis
<b>Blood (gDNA)</b> <ul style="list-style-type: none"> <li>Whole blood</li> <li>PBMC</li> <li>PBL</li> </ul>	100 ng–2 µg gDNA input  Ion Torrent™ Dual Barcode Kit 1–96	Compatible with Ion 530, Ion 540 and Ion 550 Chips  <b>Ion Torrent sequencing has a low base substitution error rate, making it ideal for immune repertoire sequencing [1]</b>	<b>Full repertoire analysis, including access to raw data, is included in Ion Reporter Software (5.16)</b>  Analysis results include several useful publication-quality visualizations and downloadable tables to facilitate secondary analysis
<b>Bone marrow</b> <ul style="list-style-type: none"> <li>BMMC</li> <li>BMA</li> </ul>	<b>Ion AmpliSeq chemistry allows for large numbers of primers in a single reaction</b>		
<b>Tissue</b> <ul style="list-style-type: none"> <li>Fresh-frozen</li> <li>FFPE</li> </ul>			
Sorted cells/cell lines			

Each assay is delivered as part of a complete solution with the Ion GeneStudio S5 platform, featuring an easy-to-use NGS workflow and the most intuitive analysis tools available.

## Powerful analysis from Ion Reporter software

- Interactive spectratyping plots make it easy to identify clonal expansion within the broader context of the repertoire
- For challenging samples with high polyclonal backgrounds, partition the repertoire by isotype, mutation rate, or diversity metrics with the click of a button to reveal repertoire features
- Automated reporting features provide detailed information on each clone, including the CDR3 sequence, SHM frequency, clone frequency, and more
- Unique automated clonal lineage analysis enables the identification of subclones based on specific sequence characteristics
- Measure and compare the frequency of a clone of interest (identified by V-gene and CDR3 NT sequence)



## Ordering information

Assay	Quantity	Cat. No.
Oncomine BCR Pan-Clonality Assay	24 reactions	A51559
	96 reactions	A51547
Oncomine BCR IGH SR Assay	24 reactions	A45483 (DNA)
		A45484 (RNA)
Oncomine IGH FR3(d)-J Assay	24 reactions	A51560
Oncomine IGH FR2-J Assay	24 reactions	A51561
Oncomine TCR Pan-Clonality Assay	24 reactions	A51562
Oncomine IGH FR1-J Assay	24 reactions	A51564
Oncomine IGHV Leader-J Assay	24 reactions	A51563
Oncomine BCR IGH LR Assay, RNA	24 reactions	A45485

## References

1. Looney TJ, Topacio-Hall D, Lowman G, Conroy J, Morrison C, Oh D, Fong L and Zhang L (2020) TCR Convergence in Individuals Treated With Immune Checkpoint Inhibition for Cancer. *Front Immunol* 10:2985.



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