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Somatic Hypermutation Analysis of Chronic Lymphocytic Leukemia Research Samples by DNA or RNA Input IGH Chain Sequencing

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ABSTRACT

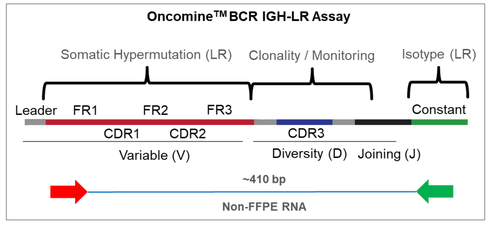
Chronic lymphocytic leukemia (CLL) is a common form of leukemia characterized by clonal expansion of neoplastic B cells and a heterogenous disease course. The accurate characterization of the somatic hypermutation (SHM) status of the IGHV gene in CLL clinical research samples is important as SHM frequency is an established prognostic biomarker. Conventionally, SHM analysis is performed via Sanger sequencing which is limited by the inability to evaluate more than one rearrangement due to mutations, multiplex constraints, and template input requirements. Ion AmpliSeq™ next generation sequencing (NGS) assays for research in IGH chain SHM are evaluated using multiple cohorts of CLL research samples. These NGS assays employ multiplex primers that target the Leader or FR1 regions of the IGHV gene and the IGHJ gene in either RNA or DNA templates. The robustness of these assays was demonstrated by an evaluation of samples in multiple labs, including comparisons between DNA and RNA input and correlation to orthogonal NGS testing.

INTRODUCTION

Oncomine™ BCR IGH LR Assay and Oncomine™ BCR IGHV SHM Assays (Leader-J/FR1-J)

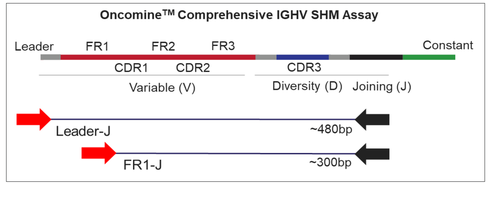
Assay Design covers CDR1, CDR2, CDR3, and CH1 domain of the constant gene with framework 1 and isotype-specific primers (FR1-C). This design enables accurate quantitation of somatic hypermutation, clonal expansion, isotype switching and identification of clonal lineages. Constant region primers are designed against all B cell isotypes and subtypes. Input requirements ranging from 25ng to 2ug of non-FFPE RNA.

Figure 1. Primer Design



Assay Design primers target the leader and FR1-J region in a separate reaction that can accurately measure clonal frequencies across B cell rearrangements. Input requirements range from 200ng to 2ug of FFPE DNA.

Figure 2. Primer Design



Qualifying SHM in Germline and CLL Research Samples

Internal Data Oncomine™ BCR-IGH LR libraries were prepared using plasmid constructs containing full length IGH chains cloned from germline and CLL research samples that were spiked into PBL total RNA background. These libraries were sequenced using the Ion™ GeneStudio S5 530 chip and analyzed using the Ion Reporter software to evaluate the ability to quantify somatic hypermutation, identify isotype, clonal structure of germline and CLL research samples.

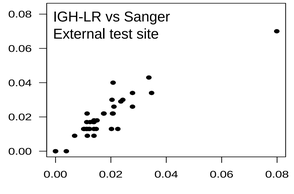
Research Sample		Expected			Observed			
Accession	SHM Status	V-Gene SHM Frequency	Isotype	Clonal Structure	V-Gene SHM Frequency	Isotype	Clonal Structure	Status
JX432218.1	Mutated	0.037	IgA1	Monoclonal	0.048	IgA1	Monoclonal	PASS
AF021966.1	Mutated	0.088	IgG2	Monoclonal	0.102	IgG2	Monoclonal	PASS
AF021964.1	Mutated	0.084	IgG1	Monoclonal	0.088	IgG1	Monoclonal	PASS
JX432219.1	Mutated	0.058	IgA2	Monoclonal	0.057	IgA2	Monoclonal	PASS
JX432222.1	Germline	0	IgG3	Monoclonal	0	IgG3	Monoclonal	PASS
AF021958.1	Germline	0	IgM	Monoclonal	0	IgM	Monoclonal	PASS
AF021967.1	Germline	0	IgD	Monoclonal	0	IgD	Monoclonal	PASS

Table 1. Indicates observed SHM levels measured using Oncomine™ BCR-IGH LR Assay is comparable to known SHM frequencies from known CLL sequences which were designed into synthetic plasmid controls.

Figure 3. Measured vs Known SHM Frequencies Dashed lines indicate 2% SHM cutoff used to distinguish germline from CLL samples. Plot compares measured and known SHM frequency using control plasmids shown in Figure 3 . V-gene SHM frequencies for constructs are calculated over entire V-gene (including leader sequence).

Correlation between Ion Oncomine™ BCR IGH LR Assay vs Sanger Sequencing

External Data Oncomine™ BCR-IGH LR SHM values were compared to those obtained by Sanger sequencing using IGH-Leader or FR1 and joining gene primer sets.



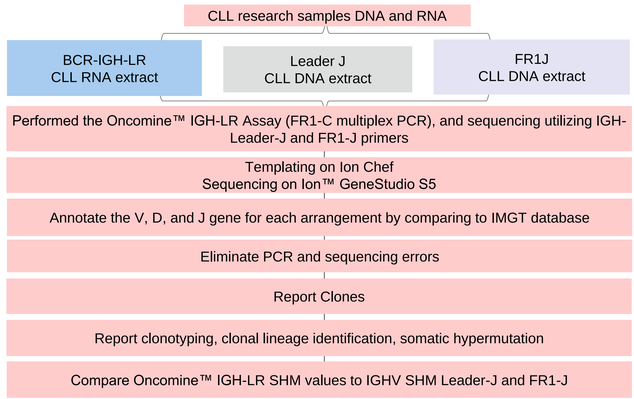
Method 1	Method 2	IGHV SHM Spearman Concordance Value
Sanger Sequencing	Oncomine BCR-IGH LR Assay (FR1-C)	0.849

Figure 4. & Table 2. High concordance when comparing the IGHV SHM frequencies between BCR IGH-LR assay with sanger sequencing.

MATERIALS AND METHODS

Experimental Design and Informatics Workflow

Figure 5. Experimental Study. Libraries were prepared using the Oncomine BCR IGH-LR Assay from total RNA, the Oncomine™ IGHV SHM Leader J and FR1-J assay from lymphoma genomic DNA or RNA. Libraries were sequenced via the Ion GeneStudio™ S5 System followed by Ion Reporter analysis to identify clonotypes and evaluate B cell clone frequencies.



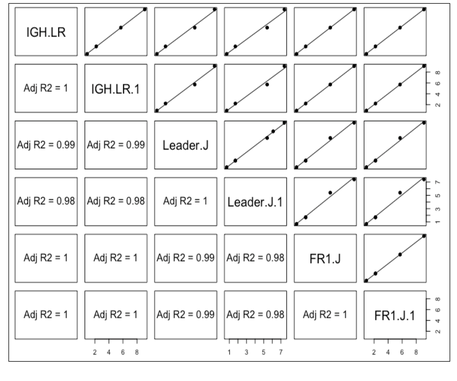
RESULTS

Correlation between Ion Oncomine™ BCR IGH LR Assay, Oncomine™ BCR IGHV Leader-J and FR1-J Assays

Cell Line	SHM Frequency measured by IGH-LR (1)	SHM Frequency measured by IGH-LR (2)	SHM Frequency measured by Leader-J Assay (1)	SHM Frequency measured by Leader-J Assay (2)	SHM Frequency measured by FR1-J Assay (1)	SHM Frequency measured by FR1-J Assay (2)
MM.1R	---	---	1.7	1.7	2.2	2.2
JVM2	0.8	0.9	0.7	0.7	0.9	0.9
BDCM	5.7	5.7	5.1	5.4	5.7	5.7
Pfeiffer	2.2	2.2	1.7	1.7	2.2	2.2
GA-10	---	---	6.1	6.1	---	---
TMM	9.1	9.1	7.4	7.4	9.1	9.1

Table 3. Both RNA and DNA input assays were able to correctly determine the SHM status of all rearrangements tested. IGHV SHM values were highly concordant between both RNA and DNA approaches. SHM values derived from FR1 targeting variable gene primers delivered concordant results compared to leader targeting variable gene primers when using DNA input across a wide range of SHM frequencies tested.

Figure 6. High concordance was observed when comparing SHM frequency values for 5 selected research cell lines that are correlated with an R^2 value of greater than 0.9 in comparison to the values derived from the IGH-LR assay.



Correlation between Ion Oncomine™ BCR IGHV Leader- J Assay vs Competitor Assay

External Data Oncomine™ BCR-IGHV Leader-J assay SHM mutation frequency rate was compared to a competitor IGH FR1 assay.

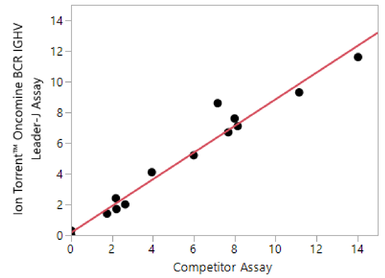


Figure 7. SHM status was concordant between NGS assays evaluated in >90% (21/24) of CLL samples tested. Mutational frequency showed excellent concordance, with an R^2=0.97, and V-gene usage was 100% concordant.

CONCLUSIONS

These results support the robustness of long-read NGS assays to quantify SHM in either DNA or RNA samples. Concordant results were shown between FR1 and Leader-targeting primers using DNA input showing the utility in both priming locations. Orthogonal testing of the Leader-J assay showed excellent concordance for mutation rate, SHM status, and stereotypy.

REFERENCES

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2. Davi, F., et al. *Leukemia* (**2020**), 34: 2545-2551; https://doi.org/10.1038/s41375-020-0923-9

ACKNOWLEDGEMENTS

R&D team, Carlsbad, CA
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The authors wish to acknowledge the external test sites who carried out orthogonal testing – Artur Kowalik (Holy Cross Cancer Center – Kielce, Poland) & Zadie Davis (Royal Bournemouth Hospital – Bournemouth, England).

The authors wish to acknowledge the external test sites who carried out orthogonal testing – R Marshall, B Pieterse, T Gerdener & T Venter (Oncolab Next Generation Diagnostics-South Africa).

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