Development and analytical validation of a novel next-generation DNA sequencing assay, the Oncomine Lymphoma Panel, to detect SNV, insertion, deletion and copy number variants in 25 Lymphoma genes in FFPE samples

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INTRODUCTION

Introduction:
Lymphomas, including diffuse large B-cell lymphoma (DLBCL), Hodgkin's lymphoma, and other lymphomas, are clinically heterogeneous: some respond well to therapy, but many fail to respond. Much of this variability in response is reported to reflect molecular heterogeneity of the tumor. Identifying somatic variants including SNVs, insertions, deletions, and copy number variations (CNVs) is important in characterizing these samples. Robust detection of variants in multiple genes using fine needle aspirate (FNA) samples, low abundance DNA, and FFPE samples is needed.

MATERIALS AND METHODS

Oncomine Lymphoma Panel for research.

We describe a next-generation sequencing research assay with 25 genes, the Ion Torrent™ Oncomine™ Lymphoma Panel, including ARID1A, ATM, B2M, BCL2, BCL6, BRAF, BTK, CARD11, CD79B, CDKN2A, CREBBP, EZH2, GNA13, HIST1H1E, KMT2D, MTOR, MYC, MYD88, PIM1, SF3B1, SOCS1, TNFAIP3, TNFRSF14, TP53, and XPO1. This panel comprises 976 amplicons in total. The assays for these genes have been optimized, and performance has been tested on control samples and on representative clinical research samples. A total of 419 genes, with optimized and verified performance, can be added to customize the panel. This panel is designed to work with 20 ng input DNA from FFPE samples and other samples.

Content selection and prioritization.

Selection of target content for specific cancer research areas was accomplished using a multifactorial scoring approach, the Gene Prioritization Framework (GPF), as follows. The framework queries, aggregates and consolidates several disease-specific genomic content sources to produce unbiased, ranked gene lists for the diseases of interest, which become the input for an assay design using these three modules:

1) The Oncomine™ Genomic Knowledgebase, a compendium of somatic variant calls, defined focal copy number alterations, and recurrent fusions.
2) The Oncomine® Reporter Knowledgebase, a biomarker-based curated compendium of diagnostic, prognostic, and therapeutic relevance.
3) The Disease-Gene Association Network, a database that organizes human diseases hierarchically and links all diseases to a set of associated genes, and then ranks genes by clinical relevance to each disease, leveraging gene-disease relationships found on DisGeNET [1].

Samples and Sample Preparation.

Genomic DNAs (gDNA) from NA12878 and NA24385 were obtained from the Coriell repository. gDNA from FFPE curls or slides. The AcroMetrix Oncology Hotspot Control (AOHC) was obtained from Thermo Fisher Scientific.

Library preparation and sequencing.

Ion AmpliSeq libraries were made either manually using the Library Kit Plus or by automated preparation on the Ion Chef™ Instrument. Libraries were templated and loaded onto Ion 530™ chips using the Ion 530 Kit on the Ion Chef Instrument . Sequencing was performed on the Ion GeneStudio S5™ System.

Sequencing data analysis.

A comprehensive bioinformatics analysis solution was developed to detect SNPs, indels, and CNVs; to annotate these variants with a wide variety of bioinformatics databases; to perform filtering for the most relevant variants; and to report on the functional interpretation of the selected variants. The analysis solution is included in the Ion Reporter software (v5.12)

Primer Design and assay optimization.

Following target selection, primers were designed to tile across the entire Coding DNA Sequence (CDS) of genes or to cover specific oncology mutational hotspots as appropriate. Primer designs were iteratively tested in highly multiplexed Ion AmpliSeq™ PCR-based NGS library preparation to yield designs with optimal performance.

Table 1. Oncomine™ Lymphoma Panel: Core and Extended Genes

Tumor type	Core Content Genes	Core Content Amplicons	Tail Genes	Tail Amplicons	Core+Tail Genes	Core+Tail Amplicons
Lymphoma	25	947	48	1642	73	2589

Table 1. Composition (number of genes and amplicons) of the Lymphoma research panel used in this study. Core Content refers to the genes/amplicons in each panel without customization. Tail Content refers to genes associated with a cancer type, but with lower GPF scores.

RESULTS

Figure 1: Oncomine tumor specific panels on ampliseq.com

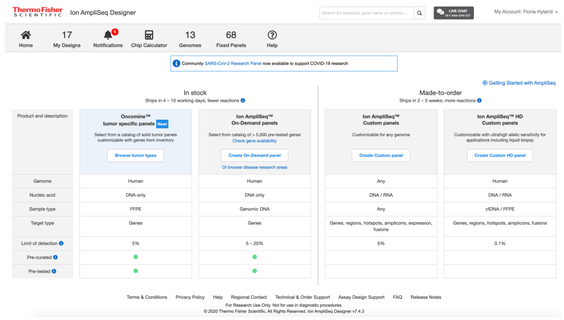


Figure 2: Panel Contents and Panel Customization on ampliseq.com

The Oncomine Lymphoma research panel contains the core 25 genes by default: it can be customized. An extended gene list is suggested, and any genes in inventory (~500 genes that are FFPE compatible) can also be added.

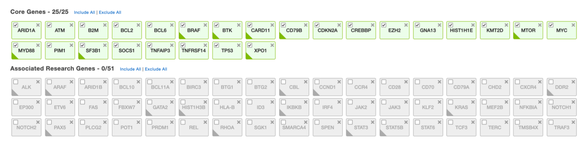


Figure 3. Gene Performance Data on ampliseq.com

Wet lab testing data are provided for all genes while a panel is being customized: shown is an example gene, ATM. An IGV view shows exon and amplicon information with a track showing amplicon coverage from wet lab testing. A track for mutational hotspots and positions relevant for oncology research is also included for relevant genes.

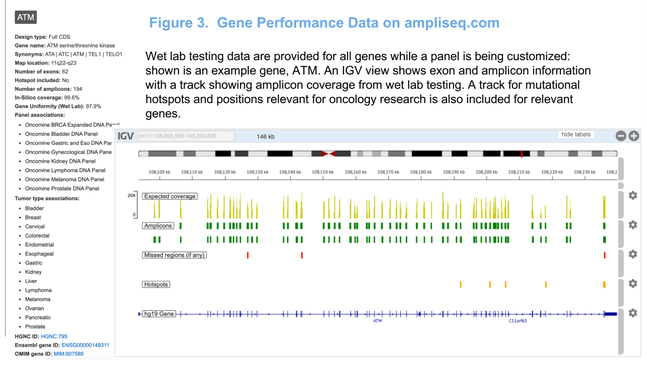
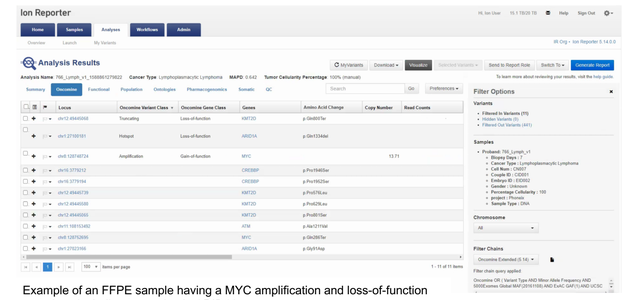
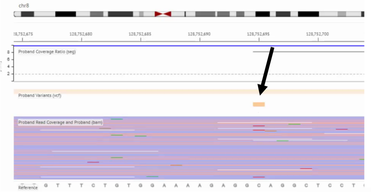


Figure 4. Example of Sample Analysis and reporting: MYC Amplification



Example of an FFPE sample having a MYC amplification and loss-of-function mutations including truncation in ARID1A.

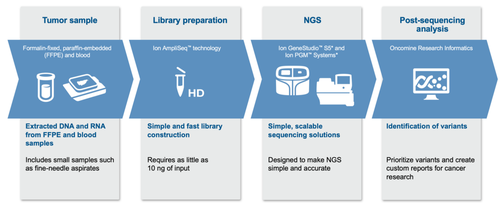


IGV view of a low frequency nonsense mutation in a Lymphoma FFPE sample

IGV view of a low frequency missense mutation in a Lymphoma FFPE sample

Amplification and Sequencing Workflow

Figure 5. Oncomine Lymphoma panel workflow on Ion GeneStudio S5



Panel customization and ordering.

An additional ten Oncomine tumor specific core panels from ten cancer research areas are available. All of these can be customized with additional suggested 'tail' genes, or adding any of the 419 genes in inventory and compatible with FFPE or non-degraded samples. Panels can be selected, customized and ordered from www.ampliseq.com.

Figure 6. Additional customizable Oncomine Tumor Specific Panels

- Ion Torrent™ Oncomine™ Bladder Panel
- Ion Torrent™ Oncomine™ Kidney Panel
- Ion Torrent™ Oncomine™ *BRCA* Expanded Panel
- Ion Torrent™ Oncomine™ Liver Panel
- Ion Torrent™ Oncomine™ Colorectal and Pancreatic Panel
- Ion Torrent™ Oncomine™ Lymphoma Panel
- Ion Torrent™ Oncomine™ Gastric and Esophageal Panel
- Ion Torrent™ Oncomine™ Melanoma Panel
- Ion Torrent™ Oncomine™ Gynecological Panel
- Ion Torrent™ Oncomine™ Prostate Panel

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RESULTS

We tested this panel on various types of input material, including control samples and FFPE samples. Uniformity, coverage, on target mapping, reproducibility, and sensitivity to detect variants were high in all cases and above established quality criteria (>90% or > 95%).
Average On-Target was 88% in FFPE samples, and average uniformity was 97%.
Finally, a coordinated analysis solution uses information about the panel and provides an integrated analysis pipeline with a simple and powerful visual interface, including variant calling, CNV detection, functional annotation, population MAF, predicted protein effect, and annotations including ClinVar, COSMIC, etc. Filtering tools utilizing this information facilitate variant prioritization.

CONCLUSIONS

An NGS lymphoma research assay with a comprehensive data analysis approach was developed and analytically validated. The system is capable of detecting both small mutations and CNVs simultaneously with high sensitivity in FFPE samples.

REFERENCES

1 Piñero J, Bravo À, Queralt-Rosinach N, Gutiérrez-Sacristán A, Deu-Pons J, Centeno E, et al. DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. Nucleic Acids Res. 2017;45: D833–D839. doi:10.1093/nar/gkw943

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