Analytical Validation of the Oncomine™ Comprehensive Assay v3 with FFPE and Cell Line Tumor Specimens in a CAP-Accredited and CLIA-Certified Clinical Laboratory

Akemi Yuki, James Chitwood, Harwinder Sidhu, Benjamin Kong, Vanessa Dasalla, James Black, Suzanne Salazar, John Williamson
Life Technologies Clinical Services Laboratory, 910 Riverside Parkway, West Sacramento, CA, 95605

INTRODUCTION

- The Oncomine™ Comprehensive Assay v3 (OCAv3) is a pan-cancer targeted NGS panel designed to detect somatic single-nucleotide variants (**SNV**), insertions and deletions (**INDEL**), copy number variants (**CNV**), and gene fusions in 161 genes.
- OCAv3 utilizes Ion AmpliSeq™ chemistry, allowing for DNA and RNA inputs as low as 10 ng of extracted material from formalin-fixed paraffin embedded (FFPE) tumor samples.
- Presented here is an analytical validation of OCAv3 at the Life Technologies Clinical Services Laboratory (LTCSL), a CAP-accredited and CLIA-certified clinical laboratory. Analytical validations provide evidence of consistently accurate and relevant sequencing results.
- As required by the Clinical Laboratory Improvement Amendments, and both CAP/AMP and New York State Somatic NGS guidelines, the analytical validation included assessment of OCAv3 performance characteristics, including analytical sensitivity (encompassing **Limit-of-Detection** (LOD) and **Sensitivity**), **Specificity**, **Accuracy** and **Precision** across a range of variants and variant types.

MATERIALS AND METHODS

Samples Tested

- A combination of FFPE tumor samples and cell lines harboring known mutations were used to evaluate OCAv3 performance characteristics
  - FFPE specimens, n=99
    - Nine different tumor types from the following locations: Colon (39), Prostate (18), Lung (17), Breast (15), Brain (3), Melanoma (2), Stomach (2) and Uterus (2), Pancreas (1)
  - Cell lines, n=11
    - Known tumor SNV, INDEL, CNV and fusions

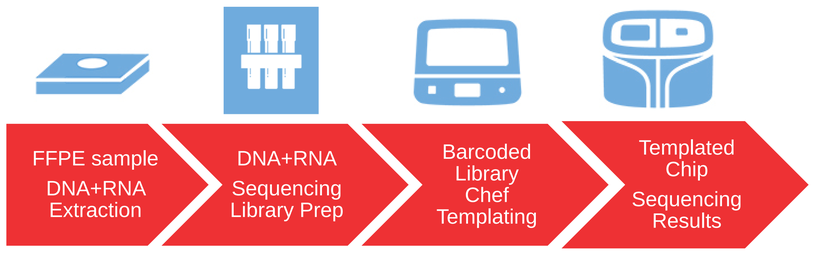

Figure 1 OCA Workflow from Sample to Sequencing

Workflow

- Following pathology assessment and microdissection, FFPE and cell lines underwent DNA and RNA extraction using a single-lysate method (RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE, AM1975, Thermo Fisher Scientific).
- 10 ng inputs (DNA and RNA for DNA and fusion libraries, respectively) were used for manual sample amplification and barcoded library synthesis (OCAv3M, A36111, Thermo Fisher Scientific)
- Automated Ion Chef™ templating of barcoded DNA and fusion library pools was performed.
- Sequencing of templated DNA and fusion library pools was accomplished using an Ion S5™ XL semiconductor sequencer on a Ion 540™ sequencing chip.

MATERIALS AND METHODS (cont.)

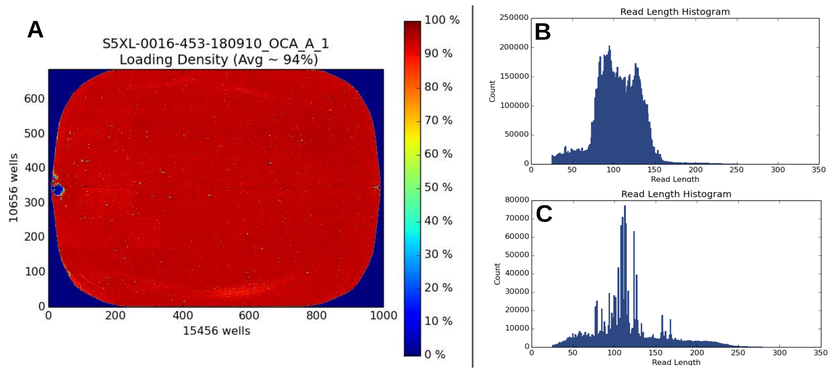

Figure 2 Representative Torrent Suite Software Sequencing Output

A) Ion 540™ Ion Sphere™ loading image (loading here is typical of a run producing 80 million sequencing reads). **B)** Histogram showing count and length of DNA library sequenced fragments. **C)** Histogram showing count and length of Fusion library sequenced fragments.

Determination of Performance Characteristics

Analytical Sensitivity – LOD and Sensitivity

- The Analytical Sensitivity (**LOD**) refers to the allelic fraction (**SNV** or **INDEL**) or copy number (**CNV**) where variants can be consistently called above non-variant alleles.
- 2 operators, 2 replicates per operator
  - n=3 cell lines (*KRAS*, *TP53*, *EGFR*) for **SNV/INDEL**
  - n=2 cell lines (*HER2*, *MET*), n=1 FFPE (Breast, *HER2*) for **CNV**

EGFR p.Glu746_Ala750del Allele Frequency

Dilution Series	Replicate 1	Replicate 2
1	87.0%	89.0%
2	22.0%	20.0%
3	6.1%	5.7%
4	4.9%	3.8%
5	3.5%	3.2%
6	1.3%	ND
7	ND	ND

Called by IR
Not called by IR
5% AF cutoff

Table 1 LOD Performance for INDEL Called by Ion Reporter™ (IR) Using a Cell Line Dilution Series

Specificity and Accuracy

- Specificity and Accuracy were assessed via comparison to orthogonal methods.
  - n=131 OCAv3 driver variants identified by orthogonal screening of 114 samples (FFPE, cell lines); 27 unique variants from 15 genes
  - Orthogonal Methodology: **SNV**▲, **INDEL**▲,**CNV**■, **Fusion**●
    - ▲ *Sanger Sequencing & Oncomine™ Focus Assay NGS*, ■ *FISH*, ● *qPCR & Pervenio™ Lung NGS*

Precision

- Precision assesses assay variability across multiple combinations of operators, instruments and library preparations.
  - **Repeatability** (intra-operator) FFPE n=4, cell line n=2
    - 3 replicates by single operator within a 24 hour period
  - **Reproducibility** (inter-operator) FFPE n=11, cell lines n=3
    - 1 replicate by 4 operators using different Ion Chef™ and Ion S5™ instrument combinations

RESULTS

Performance Characteristic	SNV	INDEL	CNV	FUSION
	> 250 reads > 5% MAF	>250 reads > 5% MAF	> 50% tumor > 7 copies	
LOD performance	>99%	>99%	>99%	N/A
Sensitivity	>99%	>99%	>99%	>99%
Specificity	>99%	>99%	>99%	>99%
Accuracy	>99%	>99%	>99%	>99%
Repeatability	>99%	90%	>99%	>99%
Reproducibility	96%	97%	>99%	95%

Table 2 OCAv3 Performance Characteristic Results for all Variant Types

CONCLUSIONS

- This analytical validation, performed in a CAP-accredited and CLIA-certified laboratory, demonstrates that OCAv3 panel produces data that is highly accurate, reproducible, sensitive, and precise for sequencing in a variety of FFPE-derived tumor tissues using low amounts of DNA and RNA input.
- All performance characteristics evaluated were at 90% and above for a wide array of biologically relevant variants
- After concluding the validation, the OCAv3 workflow was then scaled up and applied to a clinical trial. Over the course of fifteen months, more than 2,500 research specimens were sequenced. On average,180 samples were tested each month while maintaining an average turn around time of 10 days from sample receipt to report generation (excluding repeat sequencing).
- The success rate of the assay during the trial (as defined by obtaining reportable variant results from a sample) was over 95%.

REFERENCES

1. Jennings LJ, Arcila ME, et al. Guidelines for Validation of Next-Generation Sequencing-Based Oncology Panels: A Joint Consensus Recommendation of the Association for Molecular Pathology and College of American Pathologists. J Mol Diagn. 2017 May; 19(3):341-365
2. "Next Generation" Sequencing (NGS) guidelines for somatic genetic variant detection. New York State Department of Health. 2016.

ACKNOWLEDGEMENTS

- Patients and their families for the donation of samples to scientific study.
- The LTCSL leadership team: Tina Huan, Suzanne Salazar, Roxana White and Kimberly McCall allowed this study to be carried out.
- All the talented members of LTCSL.

Disclaimer

For Research Use Only. Not for use in diagnostic procedures.