A novel system that produces pre-qualified cancer NGS panels with customizable content

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INTRODUCTION

Next-generation sequencing (NGS) is the preferred method to simultaneously characterize multiple relevant genetic variants in cancer samples. In addition to pan-cancer applications, researchers are increasingly interested in cancer type specific and custom solutions. We describe a flexible system, Oncomine™ tumor specific panels (OTSP), for providing optimized, pre-tested primers for PCR-based NGS library preparation that generates cancer type specific panels with customizable content for use with DNA from FFPE tumor tissue.

A multifactorial scoring method, the Gene Prioritization Framework (GPF), was used to assign ~300 gene targets to 10 different cancer types. Fifteen to thirty genes most relevant to each particular cancer type were chosen for inclusion into core panels and an additional 15-50 genes were identified as supplemental content for each cancer type. The system is designed so that core panels can be modified by adding, removing, or substituting any of the core genes with any of the other genes inventoried in the system. The assay system uses Ion AmpliSeq™ technology with manual or automated library preparation and sequencing on the Ion Torrent™ GeneStudio™ S5 sequencing platform. Ten ng of purified DNA per library pool (20 ng total) is used as input for library preparation. An automated tumor-only analysis workflow for variant calling and sample quality reporting is provided within Ion Reporter™ Software. Streamlined access to reporting of variant relevance is enabled by Ion Torrent™ Oncomine™ Reporter Software.

In this poster, we present data on ten cancer type core panels and two customized panels from testing of commercial reference standards, reference cell lines, and solid tumor formalin-fixed paraffin-embedded (FFPE) samples.

MATERIALS AND METHODS

All reagents and equipment were from Thermo Fisher Scientific unless noted.

Samples and Sample Preparation. Genomic DNAs (gDNA) from NA12878 and NA24385 were obtained from the Coriell repository (Coriell Institute for Medical Research, Camden NJ, USA). gDNA from FFPE curls or slides was prepared using the Applied Biosystems™ MagMAX™ FFPE DNA/RNA Ultra Kit (Thermo Fisher Scientific, Waltham, MA, cat. no. A31881) and quantified using the Invitrogen™ Qubit™ dsDNA HS Assay Kit (cat. no. Q32854) with the Invitrogen™ Qubit™ 4 fluorometer (cat. no. Q33238) according to the kit instructions. The Thermo Scientific™ AcroMetrix Oncology Hotspot Control (AOHC) was obtained from Thermo Fisher Scientific (cat. no. 969056).

Oncomine™ tumor specific panels. Selection of target content for specific cancer research areas was accomplished using a multifactorial scoring approach, the Gene Prioritization Framework, 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 suing these three modules:

1) The Oncomine™ Genomic Knowledgebase, a compendium of somatic variant calls from exomes, defined focal copy number alterations from arrays, and recurrent fusions from several thousand RNAseq profiles and multiple publications such as [1, 3].
2) The Oncomine™ Reporter Knowledgebase, a biomarker-based curated compendium of diagnostic, prognostic, and therapeutic relevance in various cancer types from publicly available data sources.
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 [2].

A score is assigned to each gene-disease pair, which corresponds to the relevance of such gene for the specific disease. The framework takes as input a list of cancer types and produces, for each cancer type, a list of candidate genes, scored and ranked based on the relevance score.

MATERIALS AND METHODS - continued

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. Core panels from ten cancer research areas as well as two customized panels were ordered using the panel design user interface on www.ampliseq.com (Thermo Fisher Scientific). See Table 1 for details.

Library preparation and sequencing. Ion AmpliSeq™ libraries were made either manually using the Ion AmpliSeq™ Library Kit Plus (cat. no. 4488990) or by automated preparation on the Ion Chef™ Instrument (cat. no. 4484177) using the Ion AmpliSeq Kit for Chef DL8 (cat. no. A29024) according to the instructions provided for each kit. Manual libraries were quantified using the Ion Library TaqMan™ Quantification kit (cat. no. 4468802). Libraries were templated and loaded onto Ion S5™ 530™ chips using the Ion 530 Kit (cat. no. A30010) on the Ion Chef™ Instrument (cat. no. 4484177). Sequencing was performed on the GeneStudio™ S5 System (cat. no. A27212). The Oncomine™ Comprehensive Assay v3 (cat. no, A36111) was used to assess CNVs in FFPE tumor samples.

Sequencing data analysis. Torrent Suite™ software (v5.12.0) was used to plan sequencing runs and for primary data analysis (base calling, alignment). Coverage analysis (v5.12.0) was used to evaluate panel uniformity. Ion Reporter™ Software (v5.12.0) was used to evaluate sample variants and calculate Sensitivity and Positive Predictive Value for variants in AOHC. AOHC variants whose coverage was affected by SNPs in primers were omitted from analysis.

RESULTS

Figure 1. Panel Contents and Performance Data on AmpliSeq.com

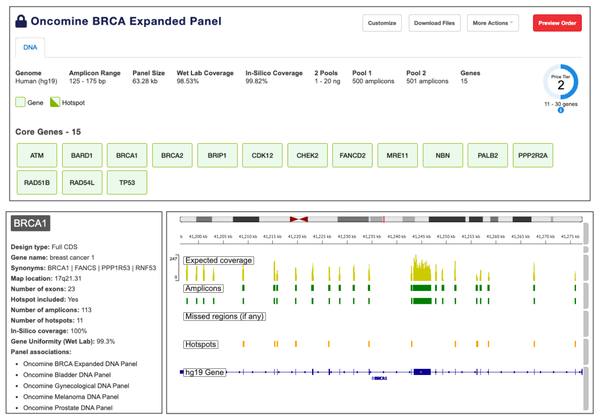

Figure 1. Screenshots from Ion AmpliSeq Designer (ampliseq.com) user interface showing the Ion Torrent™ Oncomine™ BRCA Expanded Panel. The top panel shows Core genes (highest GPF scores) and panel stats and provides access to customization and file download tools. The Customize button enables easy removal or addtion of genes, either from Tail genes (genes related to a cancer type, but with lower GPF scores) or by uploading a requested gene list. The bottom panels show wet lab testing data for an example gene, BRCA1. The bottom left panel gives gene and design information and wet lab testing results. Gene Uniformity is the % of a gene's CDS bases covered at ≥ 0.2X mean coverage of all bases in the test panel targets. The bottom right panel shows exon and amplicon level positions and coverage from wet lab testing. A track for oncology mutational hotspots and positions is also included for any gene with hotspots so that hotspot coverage can be assessed.

Table 1. Oncomine™ Tumor Specific Panels and Customized Panels in this Study

Tumor Type	Core Content		Tail Conent		Core + Tail Content	
	genes	amplicons	genes	amplicons	genes	amplicons
Bladder	25	877	37	2463	62	3340
BRCA Expanded	15	1001	25	1054	40	2055
Colorectal/Pancreatic	24	888	13	616	37	1504
Kidney	15	841	20	1041	35	1882
Liver	22	938	20	1081	42	2019
Lymphoma	25	947	48	1642	73	2589
Melanoma	29	879	30	1797	59	2676
Prostate	21	927	24	1576	45	2503
Gastric	17	910	17	1004	34	1914
Gynecologic	19	836	28	2067	47	2903
Cust. Lymphoma					72	2476
Large Custom					150	4884

Table 1. Composition (number of genes and amplicons) in the panels 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. The ten Core panels and two custom panels in this study are shown in **red**.

Figure 2. Panel Uniformity and On Target

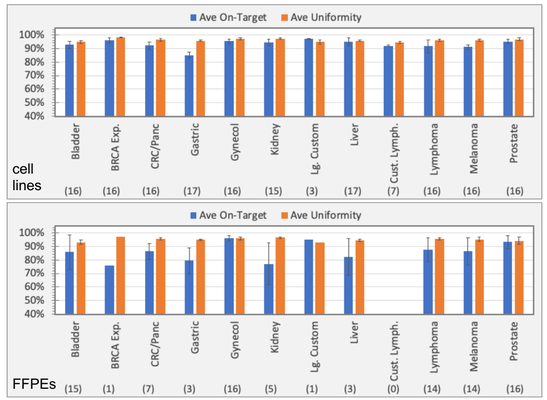

Figure 2. Panel performance with cell line controls (AOHC, NA12878, and NA24385; top panel) and FFPE tumor samples (bottom panel). Uniformity is the percentage of bases in all targets with read depth ≥ 0.2X mean read depth for a given panel. On-Target is the percentage of bases that map to a panel's target bed file. Numbers in parentheses below panel names are the library replicates across all relevant samples and library prep methods. Error bars represent ± 1 SD.

Table 2. Sensitivity and PPV for Oncology Hotspot Variants

Panel	n	# Var	SNPs				SNPs & Indels			
			Ave Sens.	SD Sens.	Ave PPV	SD PPV	Ave Sens.	SD Sens.	Ave PPV	SD PPV
Bladder	4	65	96.5%	0.8%	100.0%	0.0%	96.5%	0.8%	100.0%	0.0%
BRCA Exp.	7	17	89.9%	4.4%	100.0%	0.0%	89.9%	4.4%	100.0%	0.0%
CRC/Panc	6	59	99.4%	0.9%	98.3%	0.0%	99.4%	0.9%	98.3%	0.0%
Cust. Lymph.	3	46	95.7%	0.0%	100.0%	0.0%	95.7%	0.0%	100.0%	0.0%
Gastric	7	52	99.2%	1.0%	98.4%	0.7%	99.2%	1.0%	98.4%	0.7%
Gynecol.	4	44	98.9%	1.3%	100.0%	0.0%	98.9%	1.3%	100.0%	0.0%
Kidney	6	38	91.2%	1.1%	100.0%	0.0%	91.7%	1.1%	100.0%	0.0%
Lg. Custom	1	83	91.3%	---	96.1%	---	90.4%	---	94.9%	---
Liver	7	37	98.8%	1.4%	100.0%	0.0%	98.8%	1.4%	100.0%	0.0%
Lymphoma	4	29	93.1%	0.0%	100.0%	0.0%	93.1%	0.0%	100.0%	0.0%
Melanoma	4	58	99.6%	0.9%	100.0%	0.0%	99.6%	0.9%	100.0%	0.0%
Prostate	4	46	99.5%	1.1%	97.9%	0.0%	99.5%	1.1%	97.9%	0.0%
All Content	1	2513	97.1%	---	99.2%	---	97.1%	---	99.2%	---

Table 2. Sensitivity (Sens.) (True Positives)/(True Positives + False Negatives) and Positive Predictive Value (PPV) (True Positives)/(True Positives + False Positives) for oncology hotspots variants detected in AOHC after omission of variants in amplicons whose primers cover SNPs in the variant construct. n is the number of AOHC library reps analyzed. # Var is the number of hotspot variants mapping to the panel. Ave is average and SD is standard deviation. Variant allele frequency ranged from 5-35%.

Figure 3. Copy Number Calling with FFPE Tumor Samples

samples	13
CNV genes	7
Core Panels	10
CNV calls	38
TP	37
FN	1
FP	0
Sensitivity	97.4%
PPV	100.0%

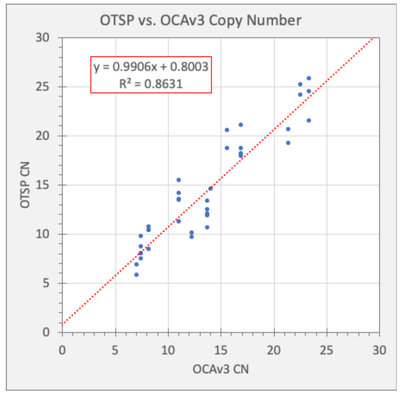

Figure 3. Thirteen FFPE tumor samples pre-tested with the Oncomine™ Comprehensive Assay v3 panel were tested with ten core oncology panels. The samples had CNVs in seven genes, allowing for 38 unique sample-panel-gene CNV calls. The data are from manual (n = 23) and automated (n = 15) library prep.

CONCLUSIONS

A novel system to provide high-quality pre-tested NGS library prep primers for pre-defined or customized cancer panels was tested on a commercial reference control, reference cell lines, and on FFPE tumor samples. Core and custom panels' average uniformity ranged from 94-98% and on-target from 85-97% on cell line controls; on FFPE tumor samples, average uniformity ranged from 93-97% and on-target from 76-96% (Figure 2). Average Sensitivity for oncology hotspot SNP and indel variants in AOHC (5-35% allele frequencies) ranged from 90-99% and average PPV ranged from 95-100%; overall Sensitivy for all targets together was 97% and PPV was 99% (Table 2). CNVs detected with these panels showed high concordance with the Oncomine™ Comprehensive Panel v3, with overall Sensitivity of 97% and PPV of 100% (Figure 3).

This system allows convenient and fast design of oncology panels from nearly 300 gene targets. As part of the design process, it is possible to view performance data of the contents by panel, gene, and amplicon to evaluate coverage of targets of interest.

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ACKNOWLEDGEMENTS

The authors would like to acknowledge our Regensburg, Germany, colleagues who synthesized, pooled, and QC'd all AmpliSeq™ primers in this study.

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