Analysis of simple and complex variants and biomarkers for comprehensive genomic profiling using a single NGS workflow for FFPE and cfDNA samples

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Abstract

Introduction: Current comprehensive genomic profiling (CGP) approaches are challenging as they require two separate eluates and a minimum of two workflows, one for DNA and another for RNA. To overcome these challenges, we developed the QIAseq Pan Cancer Multimodal Panel, a streamlined, single workflow and integrated bioinformatics for the analysis of a wide range of variants and biomarkers for oncology research.

Methods: Panel performance was verified using FFPE and cfDNA reference samples from SeraCare. Tumor nucleic acid (TNA) from FFPE samples were extracted using modified QIAGEN protocols. TNA (20–50 ng) was used as input for library construction. Libraries were constructed using the Pan Cancer Panel workflow, quantified and sequenced either on a MiSeq® or NextSeq® instrument. FASTQ files were processed with preconfigured analysis pipelines using the QIAGEN® CLC Genomics Workbench.

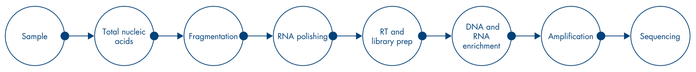
Results: All variants in reference samples were detected.

DNA: Single-nucleotide variants (SNVs) and Insertions-Deletions (InDels) were detected in cfDNA samples at variant allele frequencies (VAF) <1% and in FFPE samples down to 1% VAF. Complex variants such as the *CALR* type-1 deletion (52-bp deletion) and *FLT3* ITDs were also detected. The panel called two insertions in *CEBPA*, a GC-rich gene. Analysis of copy number variations (CNVs) showed that the panel can call CNVs at both the gene and exon levels by accurately calling six additional copies of the *EGFR*, *MET* and *MYCN* genes. Tumor mutational burden (TMB) scores were accurately called as 'low' or 'high' in the respective TMB reference samples.

RNA: All fusions, exon skipping (ES), and alternatively spliced variants (ASVs) covered by the panel were correctly called, including, but not limited to, *NTRK1*, *NTRK2*, *NTRK3*, *BCR*, *ALK* and *RET* fusions. The panel was designed to cover only one partner of all detected fusions.

Conclusion: These results provide proof-of-principle evidence that the QIAseq Pan Cancer Panel enables CGP from FFPE and cfDNA samples using a single workflow with TNA or DNA as input for both solid tumors and hematologic malignancies.

Workflow and Specifications



Workflow of the QIAseq Pan Cancer Multimodal panel (333945; UHS-5000Z). The workflow enables the use of TNA as input, followed by library preparation and enrichment. Final libraries can be sequenced on a NovaSeq® or NovaSeq platform. Read files are analyzed using the QIAGEN CLC Genomics Workbench.

Specifications of the QIAseq Pan Cancer Multimodal Panel

Panel size (region of interest)	1.4 Mb
DNA targets	523
RNA targets	56
MSI targets	26
Panel size for TMB scores	1.13 Mb
Input	10 ng TNA
Turnaround time	10 hours
Compatible sequencers	NextSeq (high output), NovaSeq
Sample multiplexing	9, 15
Sequencing setup	2 x 149
Analysis platform	QIAGEN CLC Genomics Workbench
Supporting variant classes	SNVs, InDels, CNVs, gene fusions (known and novel), transcript variants, gene expression

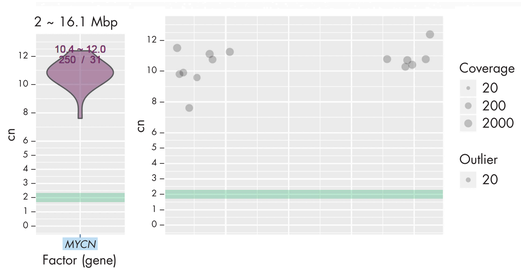
MSI: Microsatellite instability.

CNV Detection

CNV values called by the QIAGEN CLC Genomics Workbench for the three genes in sample 710-0415, which harbors six additional copies of each gene. Some regions in *EGFR* and *MET* are present in two additional copies.

Regional gene changes

Gene	Regional fold change	Regional consequence	Regional effect size
EGFR	5–8.8	Gain	Strong
MYCN	5.7	Gain	Strong
MET	5–8.7	Gain	Strong



Example of CNV output.

Solid Tumor and Onco-Heme Variants (1)

Variants called by the QIAseq Pan Cancer Multimodal Panel. Two translocations were not called since they were not covered by the panel; one mutation in *U2AF1* was not called due to ambiguously mapped reads in build hg38.

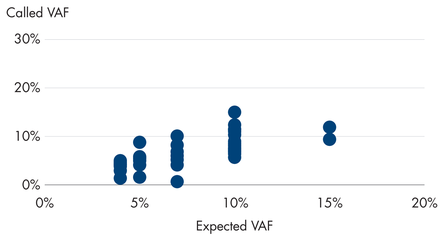
Solid tumor variants (710-0097)

ID	Nomenclature	Expected VAF	Called VAF
AKT1	c.49G>A	10%	8.9%
APC	c.4348C>T	10%	10.2%
APC	c.4666_4667insA	10%	7.8%
ATM	c.1058_1059delGT	10%	11.3%
ERBB2	c.2324_2325ins12	10%	9.2%
GNA11	c.626A>T	10%	12.0%
GNAQ	c.626A>C	10%	15.7%
KIT	c.2447A>T	10%	9.0%
MPL	c.1544G>T	10%	12.8%
PDGFRA	c.2525A>T	10%	11.9%
PIK3CA	c.1633G>A	10%	15.4%
SMAD4	c.1394_1395insT	10%	10.7%
BRAF	c.1799T>A	4%	5.0%
EGFR	c.2310_2311insGGT	4%	3.4%
EGFR	c.2236_2250del15	4%	3.7%
EGFR	c.2369C>T	4%	3.4%
FGFR3	c.746C>G	4%	3.6%
FLT3	c.2503G>T	4%	3.7%
FOXL2	c.402C>G	4%	5.0%
IDH1	c.394C>T	4%	2.8%
PDGFRA	c.1694_1695insA	4%	1.6%
PIK3CA	c.3204_3205insA	4%	4.6%
PIK3CA	c.3140A>G	4%	4.7%
RET	c.2753T>C	4%	3.4%
TP53	c.263delC	4%	3.1%

Heme variants (710-0408)

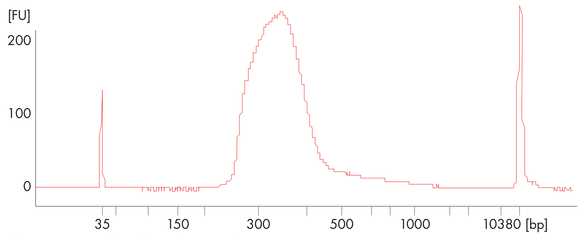
ID	Nomenclature	Expected VAF	Called VAF
ABL1	c.944C>T	10%	10.7%
ASXL1	c.1900_1922del23	10%	7.7%
ASXL1	c.1934_1935insG	10%	7.9%
BRAF	c.1799T>A	10%	10.6%
CALR	c.1092_1143del52	5%	1.9%
CBL	c.1259G>A	5%	8.9%
CBL	c.1139T>C	10%	11.0%
CEBPA	c.68_69insC	15%	9.5%
CEBPA	c.939_940insAAG	15%	12.0%
CSF3R	c.1853C>T	5%	4.7%
FLT3	c.1759_1800dup	5%	2.1%
FLT3	insGCCCC	10%	5.9%
FLT3	c.2503G>T	10%	8.8%
IDH1	c.394C>T	5%	5.3%
JAK2	c.1849G>T	5%	5.2%
JAK2	c.1624_1629delAATGAA	10%	6.9%
MPL	c.1544G>T	5%	5.6%
MYD88	c.794T>C	10%	10.6%
NPM1	c.863_864insTCTG	5%	4.9%
SF3B1	c.2098A>G	5%	4.8%
SF3B1	c.1998G>T	5%	4.3%
SRSF2	c.284_307del24	5%	4.7%

Solid Tumor and Onco-Heme Variants (2)



Concordance between called and expected VAFs (variant allele frequencies).

cfDNA Samples



Library traces prepared from cfDNA reference sample.

Examples of variants in the SeraCare cfDNA reference sample called by the QIAseq Pan Cancer Multimodal Panel

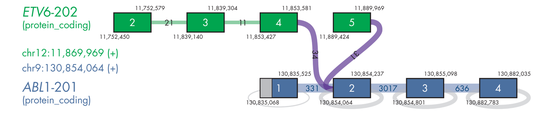
GENE ID	COSMIC identifier	Expected VAF	Called VAF	UMT
AKT1	COSM33765	1.05	1.21	821
APC	COSM13127	1.02	0.76	1588
APC	COSM18561	0.87	1.03	1747
ATM	COSM21924	1.26	0.7	1993
BRAF	COSM476	1.05	0.98	1537
CTNNB1	COSM5664	1.33	1.92	780
EGFR	COSM6224	1.23	1.4	2210

UMT: unique molecular tag; **VAF**: Variant allele frequency.

Solid Tumor and Onco-Heme Fusions

Fusions detected by the QIAseq Pan Cancer Multimodal Panel

Solid tumor fusions
CCDC6-RET
CD74-ROS1
EGFR Variant III
EGFR-SEPT14
EML4-ALK
ETV6-NTRK3
FGFR3-BAIAP2L1
FGFR3-TACC3
KIF5B-RET
LMNA-NTRK1
MET ex14 Skipping
NCOA4-RET
PAX8-PPARG1
SLC34A-ROS1
SLC45A3-BRAF
TFG-NTRK1
TMPRSS2-ERG
TPM3-NTRK1
Onco-heme fusions
BCR-ABL1
ETV6-ABL1 (transcript 1)
ETV6-ABL1 (transcript 2)
FIP1L1-PDGFRA
PCM1-JAK2



Output of the analysis pipelines showing the *ETV6-ABL1* fusion detected using two different transcripts. Numbers in purple lines show number of supporting reads used to call the respective fusions.

The fusions *MYST3-CREBBP*, *PML-RARα*, *RUNX1-RUNX1T1*, *TCF3-PBX1* are not detected since their partners are not covered by the panel.

NTRK Fusions

NTRK fusions detected by the QIAseq Pan Cancer Multimodal panel in sample 710-1031. All *NTRK* fusions were detected by targeting only the *NTRK* side of each fusion. This strategy enables the detection of both known and novel fusions.

Fusions detected by the QIAseq Pan Cancer Multimodal Panel

Fusion
TPM3-NTRK1
LMNA-NTRK1
IRF2BP2-NTRK1
SQSTM1-NTRK1
TFG-NTRK1
AFAP1-NTRK2
NACC2-NTRK2
QKI-NTRK2

Fusion
TRIM24-NTRK2
PAN3-NTRK2
ETV6-NTRK3
ETV6-NTRK3
ETV6-NTRK3
ETV6-NTRK3
BTBD1-NTRK3

TMB Score Calling

Called TMB scores are concordant with TMB scores called by other panels using the same samples.

Expected and called TMB scores using the Seraseq® TMB 7 and 20 samples

Expected TMB score	Called TMB score
7	4.8
20	17.5

Conclusions

- The QIAseq Pan Cancer Multimodal panel consolidates DNA and RNA library preparation workflows into a single, streamlined workflow.
- The panel, in conjunction with the QIAGEN CLC Genomics Workbench, delivers high sensitivity to detect low VAFs by incorporating unique molecular indexes (also known as tags).
- The low input requirements makes the panel compatible with FFPE and cfDNA samples.
- The panel has been designed to enable the analysis of a wide range of alterations including SNVs, InDels, CNVs, fusions and TMB scores.
- The panel covers a region of interest of 1.4 Mb, which is large enough to enable calling of TMB scores accurately.
- The design of the panel enables the detection of both known and novel fusions.

The QIAseq Pan Cancer Multimodal Panel is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

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Sample to Insight