QIAseq® Multimodal Analytical Technology: An Innovative NGS Workflow for Simultaneous Library Construction and Analysis from RNA and DNA

Qiong Jiang, Quan Peng, Daniel Kim, Geoff Wilt, Mohammad Ranjbar, John DiCarlo, Raed Samara, Yexun Wang, Jonathan Shaffer, Eric Lader
QIAGEN Sciences, 6951 Executive Way, Frederick, MD 21703, USA

Abstract

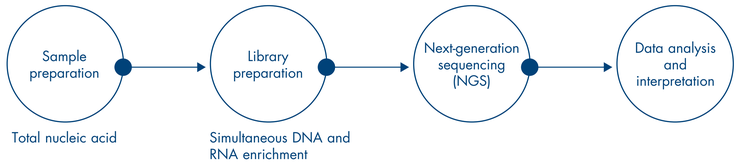
Integrated genomic and transcriptomic analysis has been proven to provide more insights in cancer research. To date, most reports show that integration happened only at the analysis stage, while genomic and transcriptomic data were still acquired in separate, sequential experiments. We have developed a novel and integrated NGS workflow, which enables the analysis of multi-omics biomarkers (DNA SNV, RNA SNV, RNA fusion and RNA expression) from the same input material.

The QIAseq multimodal workflow starts with total nucleic acid extraction from various sample types, such as FFPE tissue sections, cells and fresh biopsies. Then, total nucleic acid fragmentation, end repair, differential tagging for genomic DNA and cDNA molecules and target enrichment are performed together in a single tube. Separate DNA and RNA libraries can be generated in the final amplification step using specific PCR primers, which are then ready for quantification and sequencing.

The technology leverages single primer extension (SPE) chemistry for efficient targeted enrichment. Both DNA and RNA loci can be combined in a single enrichment panel design. The final library also contains unique molecular indexes for removing amplification artifacts from variant calling and quantitative expression analysis.

Multimodal technology realizes simultaneous library preparation and analysis from DNA and RNA, which reduces sample consumption, simplifies the process and enables confident variant calling and precise gene expression analysis.

Multimodal Workflow

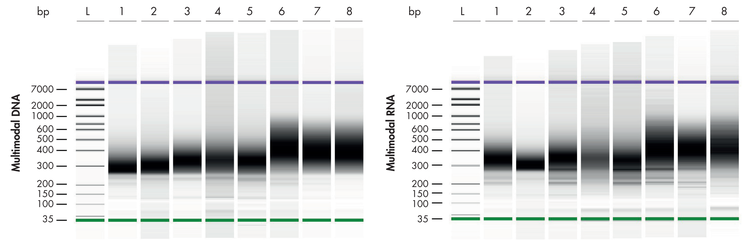


QIAseq multimodal workflow. Total nucleic acids are first isolated from blood, FFPE, serum/plasma or cells/tissues. Targeted DNA and RNA libraries are then prepared following a streamlined, consolidated workflow. After NGS, data analysis and interpretation is performed using either the QIAseq analysis pipeline or Biomedical Genomics workbench.

Methods

- Total nucleic acid was isolated from either FFPE samples or cell lines using a modified AllPrep® DNA/RNA FFPE Kit protocol (QIAGEN).
- DNA and RNA amounts were quantified using the Quant-iT™ dsDNA Assay Kit and Quant-iT RNA Assay Kit (Thermo Fisher Scientific), respectively.
- The QuantiMIZE System (QIAGEN) was used to determine FFPE DNA quality.
- Multimodal targeted DNA and targeted RNA library prep was performed with the QIAseq workflow and sequencing was performed on a MiSeq® (Illumina).

Multimodal Libraries and Primary Mapping Statistics



Bioanalyzer traces of QIAseq multimodal libraries. Libraries1–5: FFPE samples; Library 6: HD200 Quantitative Multiplex Reference Standard FFPE (Horizon); Library 7: Seraseq® FFPE Tumor Fusion Reference Material v2 (SeraCare); Library 8: HT 1080 cells. All libraries were the expected size. In addition, both the DNA and RNA libraries correlate well with the QIAseq DNA QuantiMIZE (QIAGEN) QC score (not shown). The lower the QC score, the larger the fragment size.

Mapping statistics. Primary mapping statistics are shown for the multimodal DNA and RNA libraries prepared from Library 6, Library 7 and Library 8.

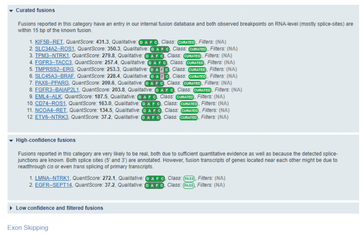
Multimodal DNA mapping metric	Library 5: FFPE tissue	Library 6: HD200 FFPE	Library 7: Seraseq FFPE	Library 8: HT 1080 cells
Read pairs total	1070367	1473747	347392	365383
Alignment pairs (%)	1020721 (97.0)	1398354 (98.0)	328000 (97.0)	346746 (97.0)
Reads with primers	1013784	1392913	325077	345505
On-target reads (%)	843455 (83.2)	1283747 (92.2)	292924 (90.1)	309364 (89.5)
Too short reads, %	16.0	6.9	8.8	9.7
Off-target, %	0.8	1.0	1.1	0.8

Multimodal DNA mapping metric	Library 5: FFPE tissue	Library 6: HD200 FFPE	Library 7: Seraseq FFPE	Library 8: HT 1080 cells
Read pairs total	773072	702337	752570	1241416
Alignment pairs (%)	678937 (96.0)	665158 (98.0)	700243 (97.0)	1151029 (98.0)
Reads with primers	645404	649281	676802	1134899
On-target reads (%)	279198 (43.3)	528003 (81.3)	532654 (78.7)	958652 (84.5)
Too short reads, %	43.1	14.8	15.2	11.0
Off-target, %	13.6	3.9	6.1	4.5

Results: HD200 Variant Analysis and Seraseq Fusion Analysis

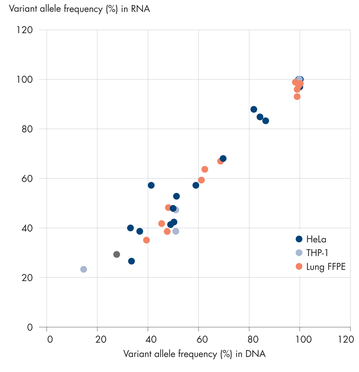
HD200 variant analysis. Variants were called using the QIAseq multimodal data analysis pipeline. All variants were called as expected. Variants were detected down to 1%.

Chromosome	Gene	Variant	Expected frequency, %	Experimental frequency, %
7q34	BRAF	V600E	10.5	7.0
7p12	EGFR	DE746 – A750	2.0	1.0
7p12	EGFR	L858R	3.0	3.2
7p12	EGFR	T790M	1.0	1.0
7p12	EGFR	G719S	24.5	24.6
12p12.1	KRAS	G13D	15.0	17.5
12p12.1	KRAS	G12D	6.0	7.4
1p13.2	NRAS	Q61K	12.5	13.1
3q26.3	PIK3CA	H1047R	17.5	17.1

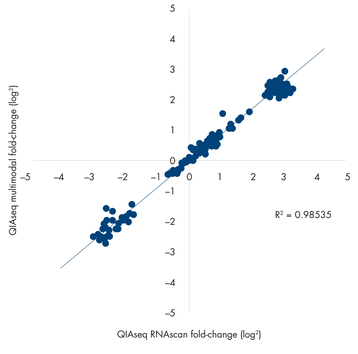


SeraCare fusion analysis. Fusions were called using the QIAseq multimodal data analysis pipeline. All fusions were called as expected, including two exon skipping.

Results: Robustly Co-analyze DNA and RNA



Variant allele frequency analysis (%): DNA vs. RNA. Variant allele frequencies were called in DNA and RNA. RNA variants represent actual expressed variants, enabling the prioritization of variants calls for follow-up assessment. In addition, RNA editing can be studied.



Robust correlation with RNA-only workflow. QIAseq multimodal workflow and QIAseq targeted RNAscan workflow (QIAGEN) were performed on THP-1 and HeLa cells. Differential expression analysis was then performed. Fold-change results between the two technologies is virtually indistinguishable demonstrating the robustness of the QIAseq multimodal workflow.

Conclusions and Future Direction

Conclusions

- QIAseq multimodal technology enables robust, simultaneous library preparation and analysis from DNA and RNA.
- QIAseq multimodal reduces sample consumption and streamlines the NGS workflow.
- QIAseq multimodal has been benchmarked using standard samples from Horizon and SeraCare.
- QIAseq multimodal works well with total nucleic acid mixtures from both FFPE samples and cell lines.
- SNV/SNP, fusion and expression analysis can be reliably analyzed from both DNA and RNA simultaneously using this integrated workflow.

Future direction

- Incorporation of additional functionality.
- Incorporation of unique dual indexing (UDI) technology.
- Integration of downstream interpretive analysis.

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Sample to Insight