



The power of one

QIAseq Multimodal's one-day workflow consolidates high-sensitivity DNA variant and RNA fusion analysis – from Sample to Insight

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Agenda

Overview of QIAseq Multimodal workflow

Detailed look: QIAseq Multimodal library construction

QIAseq Multimodal Panels: Performance

Summary



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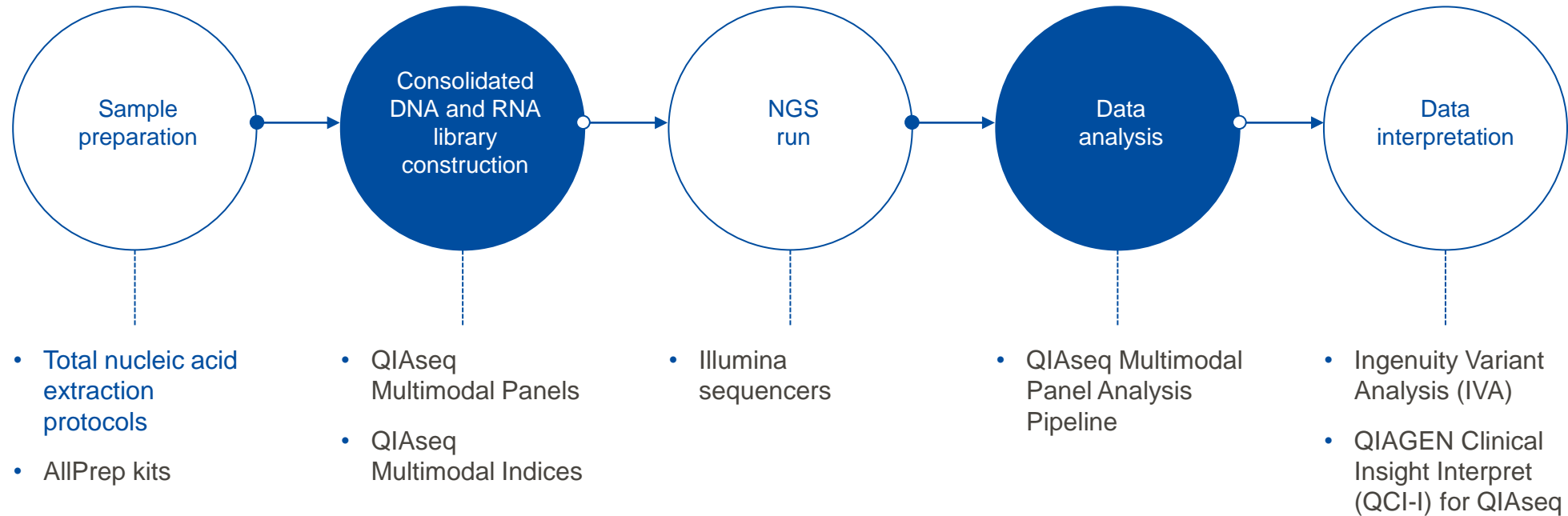
QIAseq Multimodal Panels: Performance

Summary

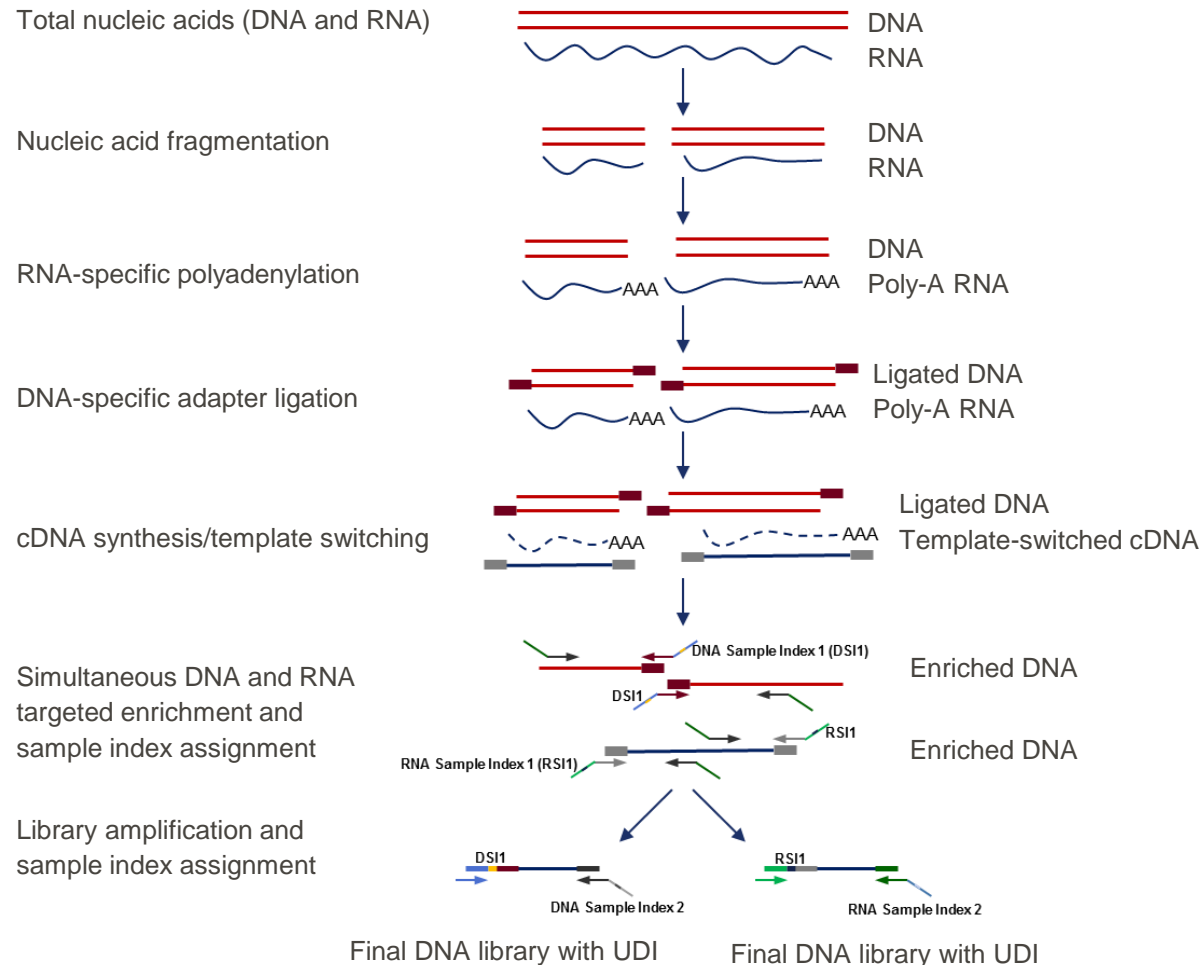


QIAseq Multimodal Panels

A Sample to Insight solution for consolidated DNA and RNA targeted library prep



QIaseq Multimodal: Consolidated DNA and RNA library construction



Sample types

- FFPE, whole blood, cells and tissues

Starting material

- DNA: 10 ng to 40 ng
- RNA: 10 ng to 250 ng
- Total nucleic acid: 10 ng to 40 ng **DNA**

Unique dual indexing (UDIs)

- Dried indices in cuttable plates
- 96 samples (192 libraries)

Total workflow time: 9–11 hours

- Total hands-on time: 3.5 hours

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Overview of QIAseq Multimodal workflow

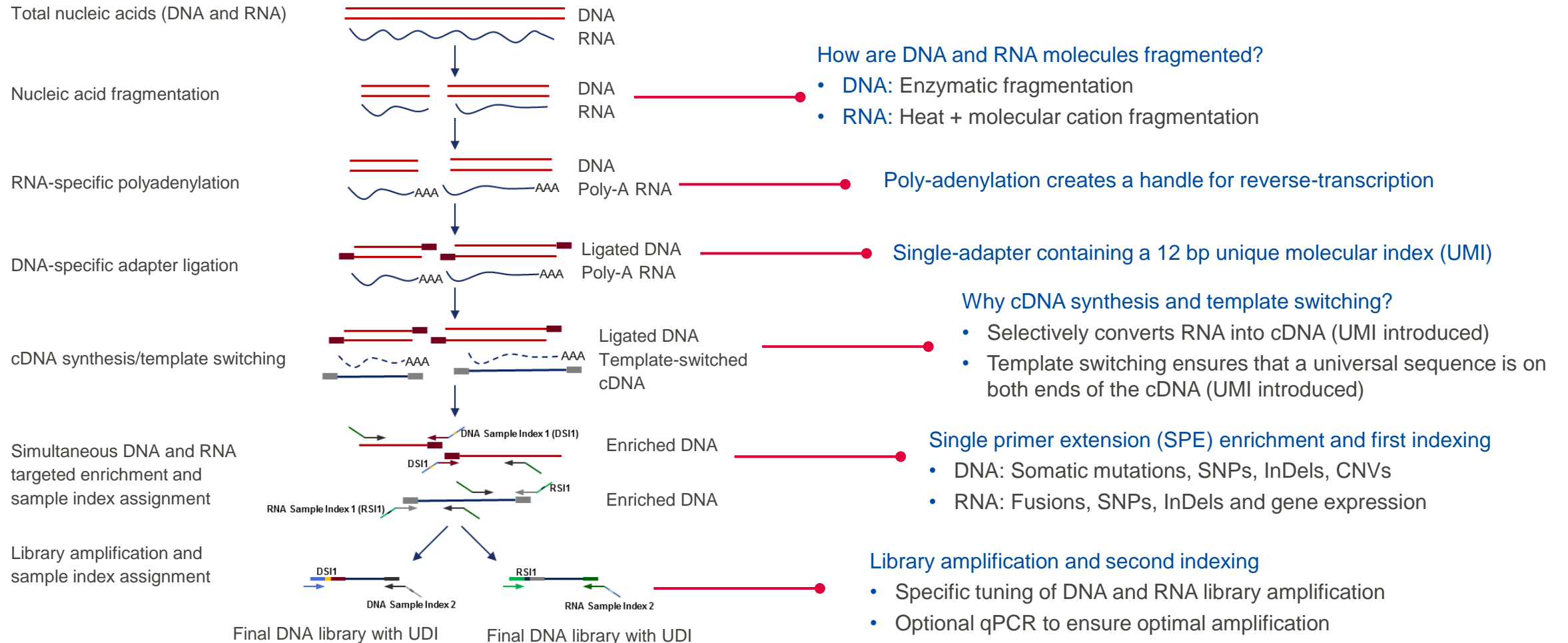
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QIaseq Multimodal: Consolidated DNA and RNA library construction



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QIAseq Multimodal Panels: Robust performance with FFPE samples

QIAseq Multimodal Panels prepare robust targeted DNA and RNA libraries from FFPE samples

Experimental overview

Samples:

- 1–5: FFPE
- 6: Horizon HD200 Quantitative Multiplex Reference Standard FFPE (Horizon)
- 7: Seraseq FFPE Tumor Fusion Reference Material v2 (SeraCare)
- 8: HT 1080 cells

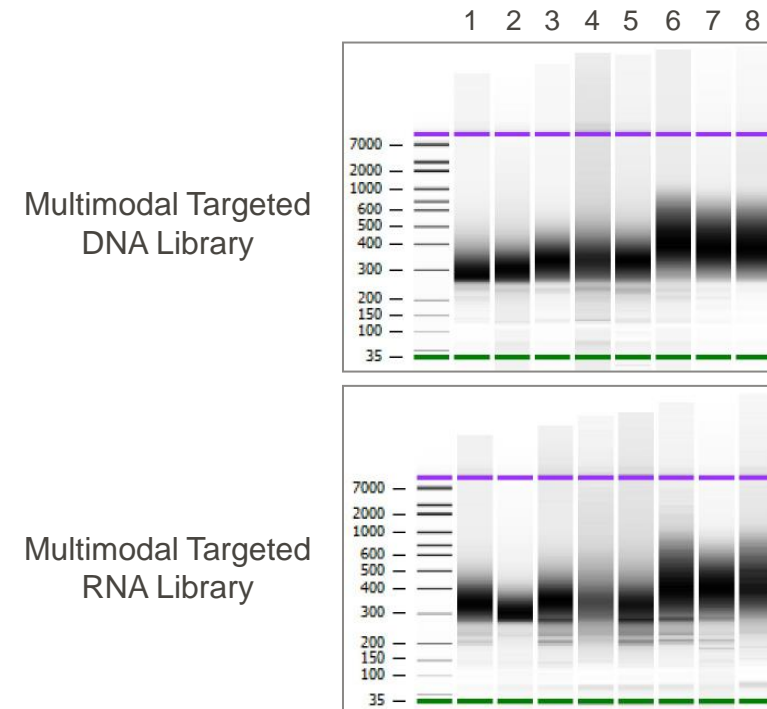
Isolation kit: QIAGEN supplementary protocol for isolation of total nucleic acid from FFPE samples

Library prep workflow: QIAseq Multimodal Panel (Lung Cancer)

Sequencing: Illumina

Data analysis: QIAseq Multimodal Data Analysis Pipeline

Bioanalyzer traces of libraries



- QIAseq Multimodal Panels prepare robust libraries, even from difficult samples.

QIaseq Multimodal Panels: Robust performance with FFPE samples (cont.)

All DNA variants were called as expected, with sensitivity down to 1%

HD200 variant analysis

Chromosome	Gene	Variant	Expected frequency (%)	Experimental frequency (%)
7q34	<i>BRAF</i>	<i>V600E</i>	10.5	7.0
7p12	<i>EGFR</i>	<i>ΔE746–A750</i>	2.0	1.0
7p12	<i>EGFR</i>	<i>L858R</i>	3.0	3.2
7p12	<i>EGFR</i>	<i>T790M</i>	1.0	1.0
7p12	<i>EGFR</i>	<i>G719S</i>	24.5	24.6
12p12.1	<i>KRAS</i>	<i>G13D</i>	15.0	17.5
12p12.1	<i>KRAS</i>	<i>G12D</i>	6.0	7.4
1p13.2	<i>NRAS</i>	<i>Q61K</i>	12.5	13.1
3q26.3	<i>PIK3CA</i>	<i>H1047R</i>	17.5	17.1

All RNA fusions were called as expected, including two exon skipping events

Seraseq fusion analysis

Curated fusions

Fusions reported in this category have an entry in our internal fusion database and both observed breakpoints on RNA-level (mostly splice-sites) are within 15 bp of the known fusion.

1. [SLC45A3–BRAF](#), QuantScore: 243.5, Qualitative: **D A F C**, Class: **CURATED**, Filters: (NA)
2. [TMPRSS2–ERG](#), QuantScore: 209.4, Qualitative: **D A F C**, Class: **CURATED**, Filters: (NA)
3. [TPM3–NTRK1](#), QuantScore: 133.9, Qualitative: **D A F C**, Class: **CURATED**, Filters: (NA)
4. [PAX8–PPARG](#), QuantScore: 130.1, Qualitative: **D A F C**, Class: **CURATED**, Filters: (NA)
5. [NCOA4–RET](#), QuantScore: 65.7, Qualitative: **D A F C**, Class: **CURATED**, Filters: (NA)
6. [KIF5B–RET](#), QuantScore: 65.3, Qualitative: **D A F C**, Class: **CURATED**, Filters: (NA)
7. [FGFR3–TACC3](#), QuantScore: 62.5, Qualitative: **D A F C**, Class: **CURATED**, Filters: (NA)
8. [FGFR3–BAIAP2L1](#), QuantScore: 45.7, Qualitative: **D A F C**, Class: **CURATED**, Filters: (NA)
9. [SLC34A2–ROS1](#), QuantScore: 42.6, Qualitative: **D A F C**, Class: **CURATED**, Filters: (NA)
10. [ETV6–NTRK3](#), QuantScore: 39.2, Qualitative: **D A F C**, Class: **CURATED**, Filters: (NA)
11. [CD74–ROS1](#), QuantScore: 34.7, Qualitative: **D A F C**, Class: **CURATED**, Filters: (NA)
12. [EML4–ALK](#), QuantScore: 18.5, Qualitative: **D A F C**, Class: **CURATED**, Filters: (NA)

High-confidence fusions

Fusions reported in this category are very likely to be real. Either both splice sites (5' and 3') are annotated, or a novel junction is supported by sufficient evidence. However, fusion transcripts of genes located near each other might be due to readthrough *cis* or even *trans* splicing of primary transcripts.

1. [LMNA–NTRK1](#), QuantScore: 194.2, Qualitative: **D A F C**, Class: **PASS**, Filters: (NA)

QIAseq Multimodal Panels: DNA and RNA variants are detected with high sensitivity

QIAseq Multimodal Panels prepare robust targeted DNA and RNA libraries from FFPE samples.

Samples:

- 40 ng Seraseq Myeloid Mutation DNA Mix (SeraCare)
- 100 ng Seraseq Myeloid Fusion RNA Mix (SeraCare)

Library prep workflow:

- QIAseq Multimodal Panel (DNA Comprehensive Cancer + RNA Human Oncology)
- QIAseq Targeted DNA Panel (DNA Comprehensive Cancer)
- QIAseq RNAscan Panel (RNA Human Oncology)





Sequencing: Illumina

Data analysis:

- QIAseq Multimodal Data Analysis Pipeline
- QIAseq Targeted DNA Panel Analysis Pipeline
- QIAseq Targeted RNAscan Panel Analysis Pipeline

Data analysis metrics

- Primer specificity, primer uniformity
- Variant calling, indel calling, fusion analysis

Workflows	DNA	RNA
QIAseq Multimodal Panel		
QIAseq Targeted DNA Panel		
QIAseq Targeted RNAscan Panel		

QIaseq Multimodal: Seraseq Myeloid Mutation DNA Mix & Myeloid Fusion RNA Mix

QIaseq Multimodal and Targeted DNA exhibit highly similar DNA variant calling

DNA variant analysis

Gene ID	Variant type	Expected VAF	QIaseq Targeted DNA VAF	QIaseq Multimodal VAF
<i>ABL1</i>	SNV	10%	12%	14%
<i>ASXL1</i>	Deletion	10%	7%	6%
<i>ASXL1</i>	Insertion	10%	11%	9%
<i>BRAF</i>	SNV	10%	15%	16%
<i>CEBPA</i>	Insertion	15%	9%	11%
<i>CEBPA</i>	Insertion	15%	11%	11%
<i>CSF3R</i>	SNV	5%	9%	8%
<i>FLT3</i>	SNV	10%	11%	11%
<i>IDH1</i>	SNV	5%	7%	10%
<i>JAK2</i>	SNV	5%	7%	8%
<i>JAK2</i>	Deletion	10%	13%	8%
<i>MPL</i>	SNV	5%	7%	10%
<i>MYD88</i>	SNV	10%	14%	16%
<i>SF3B1</i>	SNV	5%	8%	8%
<i>SF3B1</i>	SNV	5%	8%	6%
<i>SRSF2</i>	Deletion	5%	3%	3%
<i>U2AF1</i>	SNV	10%	13%	13%

QIaseq Multimodal and Targeted RNAscan exhibit highly similar RNA variant calling

RNA variant analysis

Gene ID	5' Fusion partner	3' Fusion partner	QIaseq RNAscan called? (Y or N)	QIaseq Multimodal called? (Y or N)
<i>BCR-ABL1</i>	NC_000022.11:23290413	NC_000009.12:130854064	Y	Y
<i>ETV6-ABL1 (transcript 1)</i>	NC_000012.12:11869969	NC_000009.12:130854064	Y	Y
<i>ETV6-ABL1 (transcript 2)</i>	NC_000012.12:11853561	NC_000009.12:130854064	Y	Y
<i>FIPIL1-PDGRFA</i>	NC_000004.12:53414722	NC_000004.12:54274885	Y	Y
<i>MYST3-CREBBP</i>	NC_000008.11:41937256	NC_000016.10:3851009	Y	Y
<i>PCM1-JAK2</i>	NC_000008.11:17972687	NC_000009.12:5069925	Y	Y
<i>RUNX1-RUNX1T1</i>	NC_000021.9:34859474	NC_000008.11:92017363	Y	Y
<i>TCF3-PBX1</i>	NC_000019.10:1619111	NC_000001.11:164792494	Y	Y

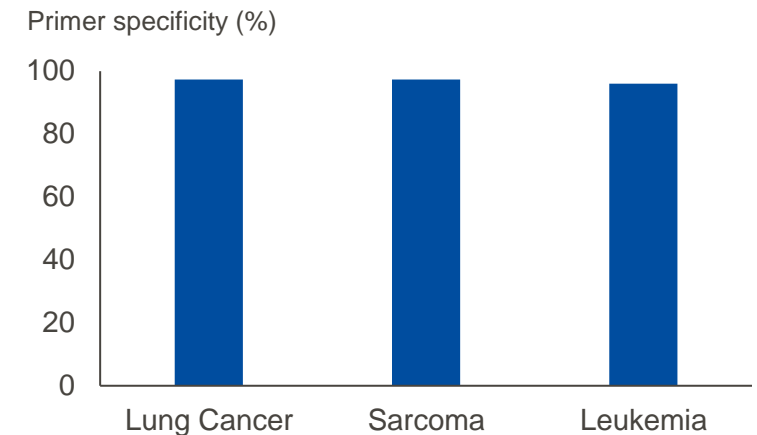
QIaseq Multimodal Panel primers: Excellent specificity and uniformity

QIaseq Multimodal Panel's primer performance matches that of QIaseq Targeted DNA Panel

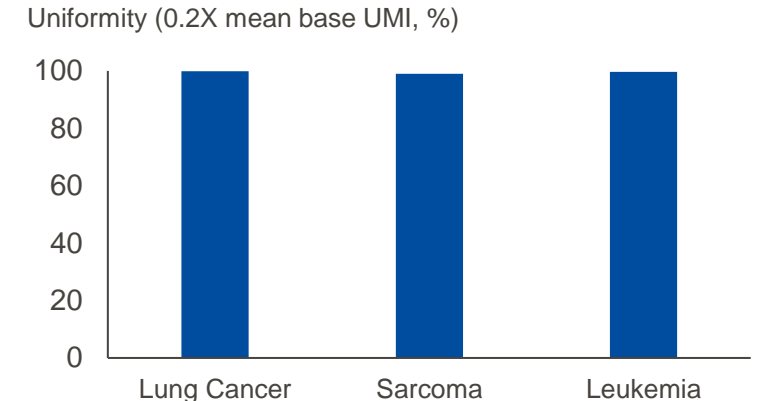
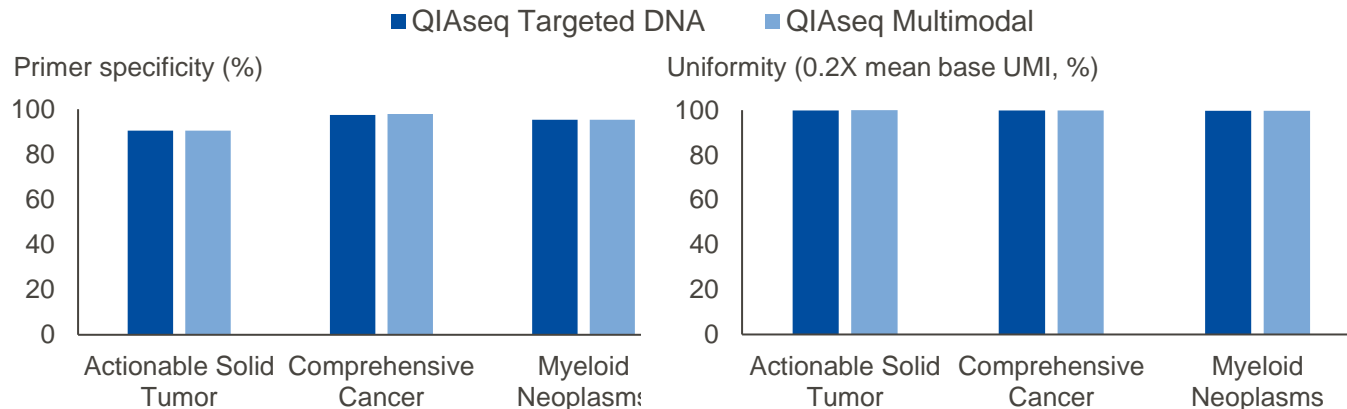
Panels tested

Panel	Associated product	Panel size (bases)
Actionable Solid Tumor	QIaseq Targeted DNA	15,160
Comprehensive Cancer	QIaseq Targeted DNA	836,670
Myeloid Neoplasms	QIaseq Targeted DNA	436,672
Lung Cancer	QIaseq Multimodal	318,059
Sarcoma	QIaseq Multimodal	836,408
Leukemia	QIaseq Multimodal	464,787

QIaseq Multimodal Panels



QIaseq Targeted DNA vs. QIaseq Multimodal Panels: Uniformity and specificity



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QIAseq Multimodal Panels: Consolidated targeted DNA and RNA library prep

Simultaneous preparation of targeted DNA and RNA libraries for robust detection of DNA and RNA variants of interest

What is QIAseq Multimodal?

- Starting with total nucleic acids or separate DNA & RNA eluates, QIAseq Multimodal provides all reagents to simultaneously enrich for DNA and RNA regions of interest
- Consolidates workflows, resulting in sample, time and money savings

What is the starting material?

- 10–40 ng (DNA) and 10–250 ng (RNA)
- FFPE, whole blood, cells and tissues

How long does the workflow take?

- 9–11 hours (3.5 hours hands on time)

What panels are available?

- Cataloged panels: Lung Cancer, Sarcoma and Leukemia
- Custom

What type of sample indices are available?

- Unique dual indices (UDIs)

What data analysis options are available?

- GeneGlobe Data Analysis Center and CLC Genomics Workflow

What data interpretation options are available?

- Ingenuity Variant Analysis (IVA) tool or QIAGEN Clinical Insight Interpret (QCI-I) for QIAseq

The background of the slide features a blue-tinted photograph of a laboratory. In the foreground, a clear plastic pipette is shown dispensing liquid into a clear microcentrifuge tube. The background is slightly out of focus, showing a white label with the word "QUALITY" and other blurred text.

Thank you for your attention.



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