Pan-Cancer 6-Fusion panel FFPE RNA (HD834) reference standard.

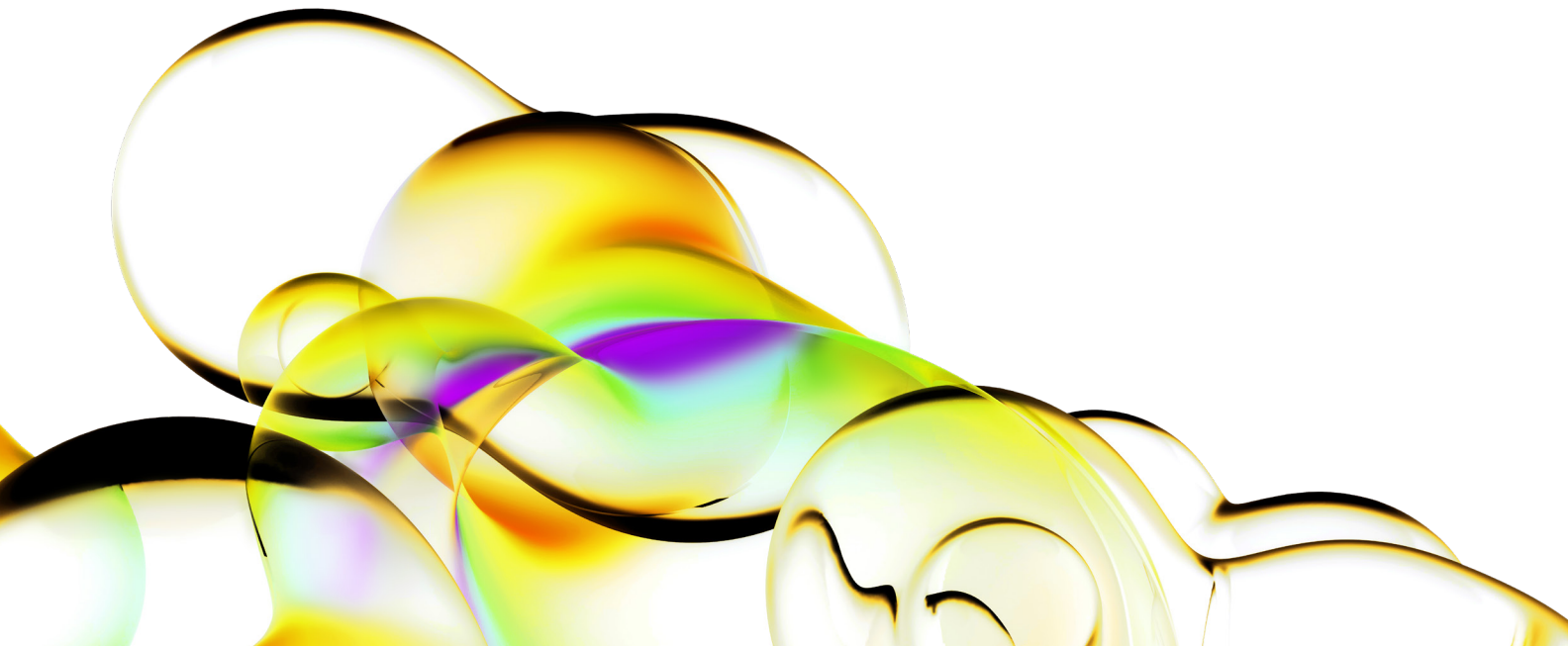
Defined FFPE control material, containing 6 clinically-relevant, cell-line derived, kinase fusion RNA biomarkers, in the complexity of the cell transcriptome.

Introduction

Revvity's Mimix™ Pan-Cancer 6-Fusion Panel FFPE RNA Reference Standard is a highly characterized, clinically relevant, cell-line derived quality control material used to assess the performance of targeted NGS RNA-seq, endpoint reverse-transcription PCR (RT-PCR), RT-qPCR and RT droplet digital (RT-ddPCR) assays aimed at detecting gene fusions.

Each 15 µM section contains a multiplexed blend of formalin-fixed, paraffin-embedded (FFPE) cell lines verified to contain *TPM3-NTRK1, QKI-NTRK2, ETV6-NTRK3, EML4-ALK, CCDC6-RET* and *SLC34A2-ROS1* fusions (Table 1).

5' Partner	5' Partner Chromo- some	3' Partner	3' Partner Chromo- some
TPM3 (Exon 7)	1	NTRK1 (Exon 10)	1
QKI (Exon 6)	6	NTRK2 (Exon 14)	9
ETV6 (Exon 5)	12	NTRK3 (Exon 15)	15
EML4 (Exon 13)	2	ALK (Exon 20)	2
CCDC6 (Exon 1)	10	RET (Exon 12)	10
SLC34A2 (Exon 4)	4	ROS1 (Exon 32)	6



Description

Targeted NGS, RT-PCR, RT-qPCR and RT-ddPCR platforms offer researchers tremendous potential for profiling biomarkers in tumor samples. However, data interpretation can be challenging due to inherent variabilities in samples, RNA extraction procedures, RNA quantification and platform biases. This can result in low confidence results and a potential failure to detect biomarkers.

To aid in the development, validation and routine monitoring of these assays, Horizon has developed a targeted *TPM3-NTRK1, QKI-NTRK2, ETV6-NTRK3, EML4-ALK, CCDC6-RET and SLC34A2-ROS1* verified cell-line multiplex in an FFPE format. This renewable and highly-characterized biologically-relevant reference material contains cell-lines that were either clonally derived from a fusion background or engineered to express the fusion of interest.

The utilization of clonal cell populations derived from single-cell dilution and controlled manufacturing environment, ensure reproducible amplifiable levels of each fusion mRNA allowing for a consistent external quality control.

Achieve end-to-end process validation with a homogeneous cell suspension that yields a consistent and reproducible quantity of RNA.

- Evaluate your workflow integrity from pre-analytical RNA extraction through to fusion detection
- Optimize and validate new targeted RNA panels and routinely monitor the performance of your assay
- Assess the performance of your RNA-seq, RT-PCR, RT-qPCR or RT-ddPCR assays aimed at detecting *TPM3-NTRK1, QKI-NTRK2, ETV6-NTRK3, EML4-ALK, CCDC6-RET and SLC34A2-ROS1 fusions*
- Determine analytical variability and reproducibility between platforms, laboratories, operators and assays
- Ensure confidence in your clinical workflow, from RNA extraction to analysis

Background

Recent advances in transcriptomic profiling in the field of clinical oncology have greatly enhanced our understanding of both the role and prevalence of kinase fusions across many different cancer types, while also highlighting the importance of novel fusion detection (Stransky, et al., 2014; Zehir, et al., 2017).

Identifying novel gene fusions with traditional diagnostic methods, such as FISH and targeted RT-qPCR can be challenging due to the promiscuous nature of certain kinase fusions, some of which have been identified with multiple fusion partners e.g., *ALK* has 22 identified fusion partners, while the *NTRK 1, 2 & 3* genes have been identified with over 60 different partners (Illumina Inc., 2019, Kummar, S. & Lassen, U.N., 2018; Stransky, et al., 2014).

Fusion detection from DNA is further complicated by the fact that fusions can often have multiple DNA breakpoints, that are often in large intronic regions, making clinical testing impractical and expensive.

Fusion sequences are, however, much more conserved at the RNA level, thus allowing RNA profiling to overcome the challenges posed by the variability inherent in structural fusion variants (Illumina Inc., 2019). With the advancement of transcriptomic profiling in clinical oncology comes a strong need for high quality, well-characterized and reproducible fusion RNA controls, especially with respect to the *ALK, RET, ROS* and *NTRK fusions*.

All 6 fusion biomarkers in HD834 are listed in international and/or local guidelines for non-small cell lung carcinoma (NSCLC) (Lindeman, Neal I. et al., 2018) and other solid tumors and are included in several targeted oncology NGS screening panels, including Illumina's™ TruSight™ Oncology 500 assay (Illumina™ Inc., 2022) and the ArcherDX™ FUSIONPlex™ Dx assay (INVITAE™ Corp., 2022).

Fusion biomarker expression

Fusion biomarker expression is assessed in FFPE RNA extracted from HD834 sections by RT-ddPCR using SuperScript™ IV (SSIV) reverse-transcriptase (RT) (Invitrogen™). Triplicate ddPCR analysis for each biomarker is performed using fusion specific probes on the Bio-Rad™ QX200 ddPCR platform. Fusion expression (Copies/ng RNA) is then calculated for each fusion (Figure 1).

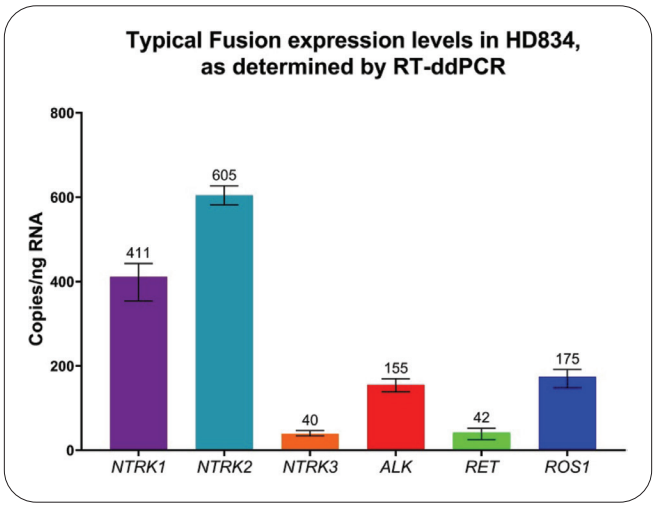


Figure 1: HD834. (Mean expression form FFPE RNA sections from 3 batches (mean, with max/min error bars shown).

DV_{200} for assessing FFPE RNA integrity

Degraded FFPE RNA typically lacks the distinctive 18S and 28S rRNA peaks, making RNA Integrity (RIN) values less relevant and reliable as quality metrics.

For RNA-seq library preparation, the average fragment size of RNA has been shown to be a better determinant of RNA Quality (Illumina™, Inc., 2016). The DV_{200} score, developed by Illumina™, is calculated from an RNA electropherogram trace and reports the percentage of RNA fragments greater than 200 nucleotides (nt) present in a sample (Illumina™, Inc., 2016).

DV_{200} correlates strongly with both library yield and gene expression counts from RNA-seq analysis of FFPE RNA (Illumina™, Inc., 2016; Matsubara, et al., 2020). FFPE RNA with a DV_{200} ≥65% is regarded as being high quality (Illumina™, Inc., 2016).

Horizons FFPE manufacturing process has been optimised to deliver FFPE RNA of consistent quality and integrity that is both representative of clinical FFPE material and suitable for high-quality NGS RNA-seq library perpetration, with typical DV_{200} ≥65% (Figure 2).

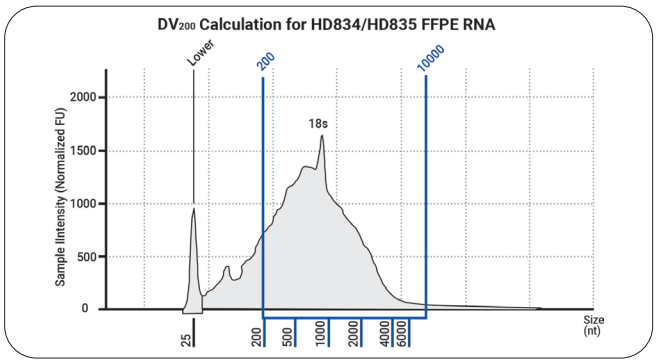


Figure 2: Typical TapeStation electropherogram trace for HD834/HD835 FFPE RNA, showing DV_{200} region (200–10000 nt) in blue (measured DV_{200}=83%), as measured with the High Sensitivity RNA ScreenTape® Assay (Agilent™).

Technical data

Format: FFPE
Genes Covered: *TPM3-NTRK1, QKI-NTRK2, ETV6-NTRK3, EML4-ALK, CCDC6-RET and SLC34A2-ROS1*
Fixation Method: 10% Formalin
Section Size: 15 µM

Product information

Intended Use: For assay validation and routine monitoring of assay performance. (Research Use Only)
Unit Size: 1 x 15 µM FFPE Section

General information

Storage: 4 °C
Expiry: See all product shelf-life information

Quality control

Extractable RNA/Expected Yield: ≥ 100 ng RNA per section using the Maxwell RSC™ RNA FFPE Purification Kit (Promega™)
RNA Quantification: Qubit™ RNA HS Assay (Thermo Fisher Scientific™)
Fusion Biomarker Expression: Expression of *TPM3-NTRK1, QKI-NTRK2, ETV6-NTRK3, EML4-ALK, CCDC6-RET and SLC34A2-ROS1* RNA transcripts confirmed by fusion specific RT-ddPCR using the QX200 ddPCR System (Bio-Rad™).
RNA Integrity: Expected DV_{200} ≥65% assessed using TapeStation™ High Sensitivity RNA ScreenTape™ Assay (Agilent™)

References

1. Illumina, Inc., 2016. Evaluating RNA Quality from FFPE Samples (Technical Note). [Online] Available at: https://www.illumina.com/
2. Illumina Inc., 2019. Comprehensive NTRK Fusion Detection, Agnostic of the Fusion Partner for Optimal Identification of Rare Genomic Events. [Online] Available at: https://www.illumina.com/
3. Illumina Inc., 2022. TruSight Oncology 500 Portfolio. [Online] Available at: https://www.illumina.com/
4. Invitae Corp., 2022. FusionPlex Dx. [Online] Available at: https://archerdx.com/
5. Kummar, S., Lassen, U.N. TRK Inhibition: A New Tumor-Agnostic Treatment Strategy. Targ Oncol 13, 545–556 (2018).
6. Lindeman, Neal I. et al., 2018. Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors. Journal of Thoracic Oncology, Volume 13, Issue 3, 323 – 358
7. Matsubara, T. et al., 2020. DV200 Index for Assessing RNA Integrity in Next-Generation Sequencing. BioMed Research International, vol. 2020, Article ID 9349132, 6 pages.
8. Stransky, et al., 2014. The landscape of kinase fusions in cancer. Nat Commun, Volume 5, p. 4846.
9. Zehir, A. et al., 2017. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med, Aug 4; 23(6), pp. 703-713.

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com

1398066 (1221615)