AACR 2018

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Redefining Standards:

Gain Confidence in your Liquid Biopsy Assay with cfDNA Controls

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Horizon's Reference Standards

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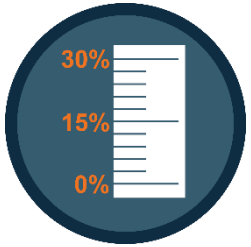
Cell line-derived, mimics patient genetics: Variants presented in relevant genomic complexity

Multiple sample formats mimicking real samples



Range of allelic frequencies

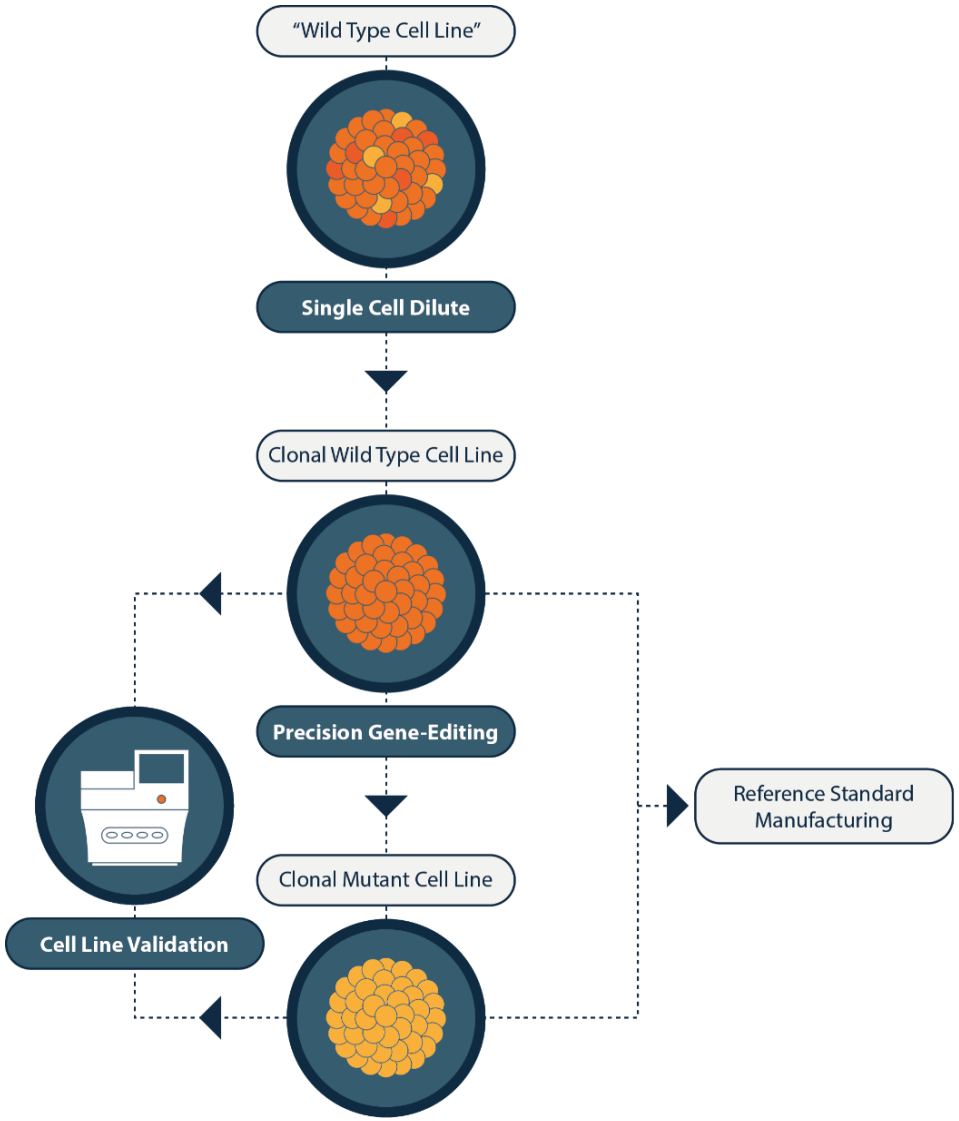
Quality-controlled and validated

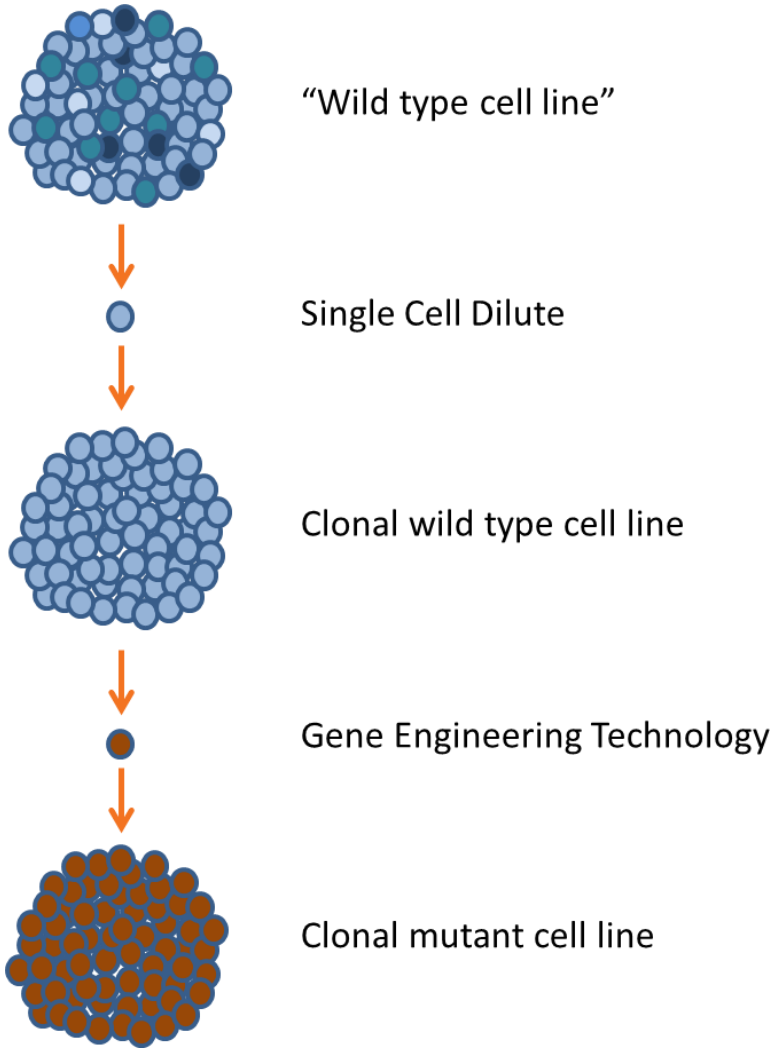
Prepared under a certified quality management system

ISO 9001

ISO 13485

Horizon's unique approach to Reference Standards





OncoSpan

Validate large gene panels and exomes with the world's largest oncology Reference Standard

- **386 variants** across 152 cancer genes
- Batch-specific NGS data (VCF) available
- Supported by our assay validation and QC tool **OncoMatic**

Cell-free DNA

Ensure the accuracy of your liquid biopsy assay from DNA extraction to analysis

- ***EGFR* Multiplex Set** (10 variants)
- **Multiplex I** in buffer/synthetic plasma
- **Structural Multiplex** (CNVs, INDELs, fusions)

Multi-Gene Multiplexes

Assess and control multi-gene NGS and PCR assays

- **Quantitative Multiplex** (11 variants)
- **Structural Multiplex** (CNVs, INDELs, fusions)
- **Tru-Q** (44 variants)

Single-Gene Multiplexes

Qualify the data from single-gene NGS and PCR assays

- ***BRCA*** (13 variants)
- ***EGFR*** (5 variants)
- ***KRAS*** (6 variants)

RNA Fusions

Optimize your whole workflow with our 5-Fusion FFPE RNA Multiplex control containing ***ALK, RET, ROS1, NTRK1*** and ***NTRK3*** fusions

Base Seq

Monitor the performance of **single SNP PCR assays** and **Sanger sequencing** with matched wild type in gDNA and FFPE format

Genome in a Bottle

Investigate the impact of formalin fixation with downloadable WGS truth set

Custom

We can **customize according to your needs and specifications**. Please get in touch!

gDNA • RNA • formalin-compromised DNA • FFPE • cell-free DNA • synthetic plasma

How to use Horizon's Reference Standards in NGS workflows

Plasma cfDNA
FFPE DNA
FFPE RNA

Tumor Sample

DNA Extraction

DNA Quantification

cfDNA
gDNA
FcDNA

Library Preparation

Sequencing

Bioinformatics

Analysis

EGFR Multiplex cfDNA Reference Standard Set

Cat. No: HD825

- **10 variants:** EGFR T790M, L858R, ΔE746-A750, L861Q, G719S, C797S, S464L, G465R, V769-D770insASV, S768I
- **0.1%, 1%** and **5%** allelic frequency, plus **wild type** (set of 4)
- **Average fragment size:** 160 base pairs
- Available in **buffer**

Tested on Cobas®, Therascreen, Idylla™ and BioRad!

Application: For **NGS** panel validation and **PCR-based assays** using liquid biopsies

Parameters that can be assessed: Assay **sensitivity**, **specificity** and **limit of detection**

Multiplex I cfDNA Reference Standard Set

Cat. No: HD780, HD816, HD817

- 8 variants across 4 genes:
  - ✓ ***EGFR*** L858R, ΔE746-A750, T790M, V769-D770insASV
  - ✓ ***KRAS*** G12D
  - ✓ ***NRAS*** Q61K, A59T
  - ✓ ***PIK3CA*** E545K
- **0.1%, 1%** and **5%** allelic frequency, plus **wild type** (set of 4)
- **Average fragment size:** 160-170 base pairs
- Available in **buffer** and in **synthetic plasma**

Applications: For **NGS** panel validation and **PCR-based assays** using liquid biopsies

Parameters that can be assessed: Assay **sensitivity**, **specificity** and **limit of detection**

Whole process control: Test for **DNA extraction efficiency** with our synthetic plasma control

Multiplex I cfDNA Reference Standard Set

Calculating Limit of Detection (LOD) and False Positive Error Rates

Gene	Variant	Allelic Frequency			
		5%	1%	0.1%	0% (WT)
EGFR	L858R	5.0	1.0	ND	ND
EGFR	ΔE746-A750	4.9	0.9	ND	ND
EGFR	T790M	4.9	1.1	ND	ND
EGFR	V769-D770ins	5.0	1.0	ND	ND
KRAS	G12D	5.1	1.0	ND	ND
NRAS	Q61K	4.9	0.9	ND	ND
NRAS	A59T	5.2	1.1	0.7	0.7
PIKC3A	E545K	5.0	1.0	ND	ND

ND = Not Detected

In this example data set, the Reference Standard informs the user that:

- Their reliable ***limit of detection*** for this cfDNA assay is 1% AF
- They are calling a ***false positive*** for NRAS A59T

This allows pipeline optimisation and confidence in results when handling real patient samples!

Benefits of using our synthetic plasma control

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Real (Human) Plasma

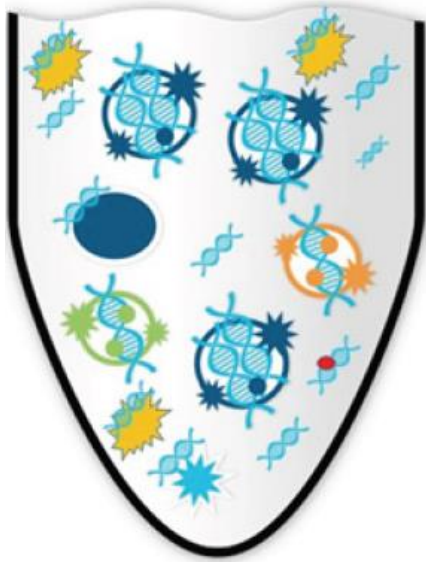
Variable quantity and concentrations ☹

Lot-to-lot variability ☹

Irregular supply ☹

Contamination with other analytes/genomic DNA ☹

cfDNA degradation: time-limited storage ☹



Horizon Synthetic Plasma

Defined volume and concentrations ☺

Lot-to-lot stability ☺

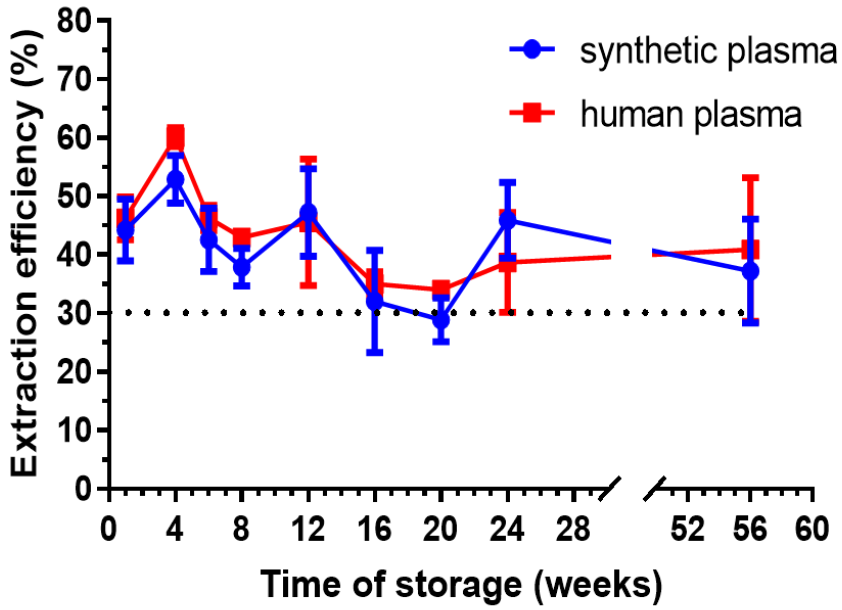
Reliable supply ☺

No interfering analytes/genomic DNA ☺

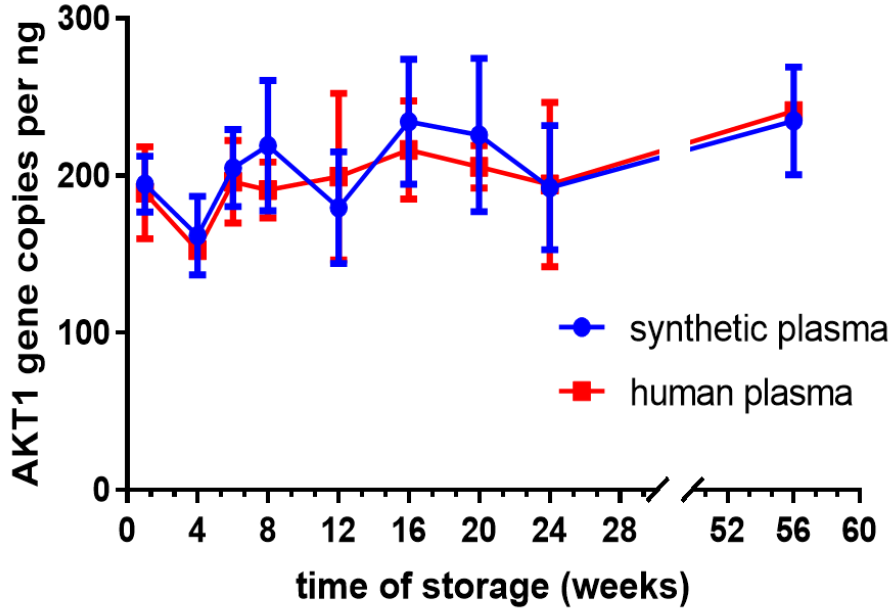
Long term stability of cfDNA beyond 24 months ☺

Performance of synthetic plasma compared to human plasma

cfDNA recovery



Gene copy number detection



- 400 ng of cfDNA was spiked into 1 ml of either human or synthetic plasma, and stored at -80˚C
- cfDNA was extracted by using Circulating Nucleic Acid kit (Qiagen), extraction efficiency was measured with Qubit BR Reagents (Molecular Probes)
- Total *AKT1* gene copies were quantified by ddPCR (Biorad)

Structural Multiplex cfDNA Reference Standard

Cat. No: HD786

- 9 variants across 8 genes, 5% allelic frequency
- **Single nucleotide variants (SNV) in GC-rich/low regions**: ***GNA11*** Q209L, ***AKT1*** E17K, ***PIK3CA*** E545K
- **Indels:** ***EGFR*** ΔE746-A750, V769-D770insASV
- **Fusions:** ***SLC34A2-ROS1***, ***CCDC6-RET***
- **Copy number variations (CNVs)**: ***MET*** (4.5x), ***MYC-N*** (9.5x)
- **Fragment size:** 160 base pairs

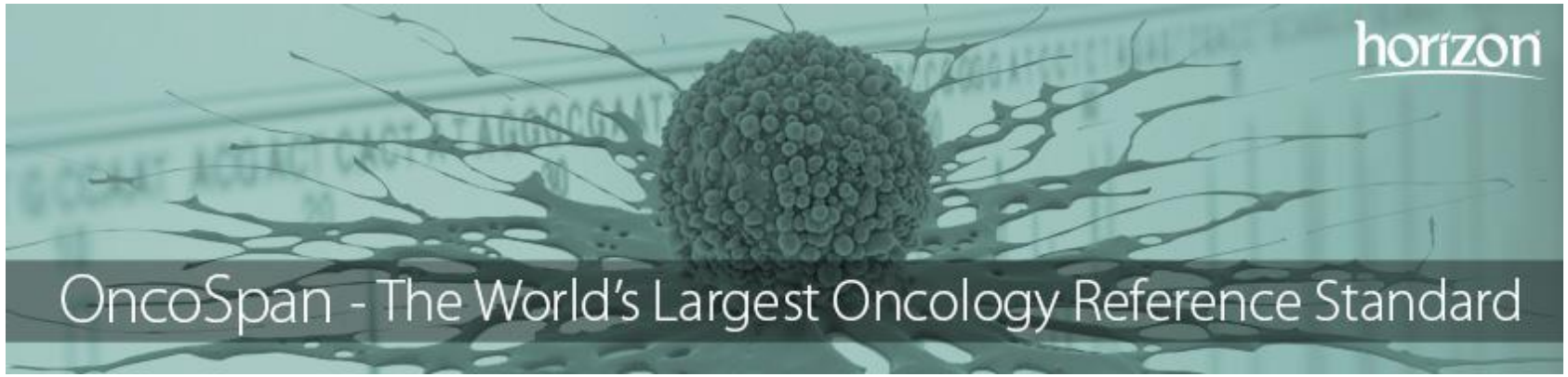
Also available in gDNA (HD753) and FFPE (HD789)

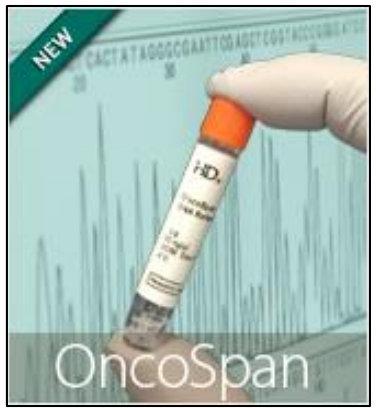
Applications: For **NGS** panel validation and **PCR-based assays** using liquid biopsies

Parameters that can be assessed: Assay **sensitivity, structural variants, GC-rich/low regions**

What’s new?

OncoSpan gDNA





- ***386 variants*** across **152 oncology genes**, confirmed by **NGS**
- **25 variants** across **16 oncology genes**, validated by **ddPCR**
- 356 SNPS and 30 INDELs (24 deletions and 6 insertions ranging from 2 to 16 bp), allelic frequencies from 1% to 100%
- ***Batch-specific NGS data*** (VCF and BED files)
- Supported by a bespoke companion NGS quality control tool, ***OncoMatic***, for faster and more convenient assay validation → perfect for routine monitoring

5-Fusion Multiplex FFPE RNA Reference Standard

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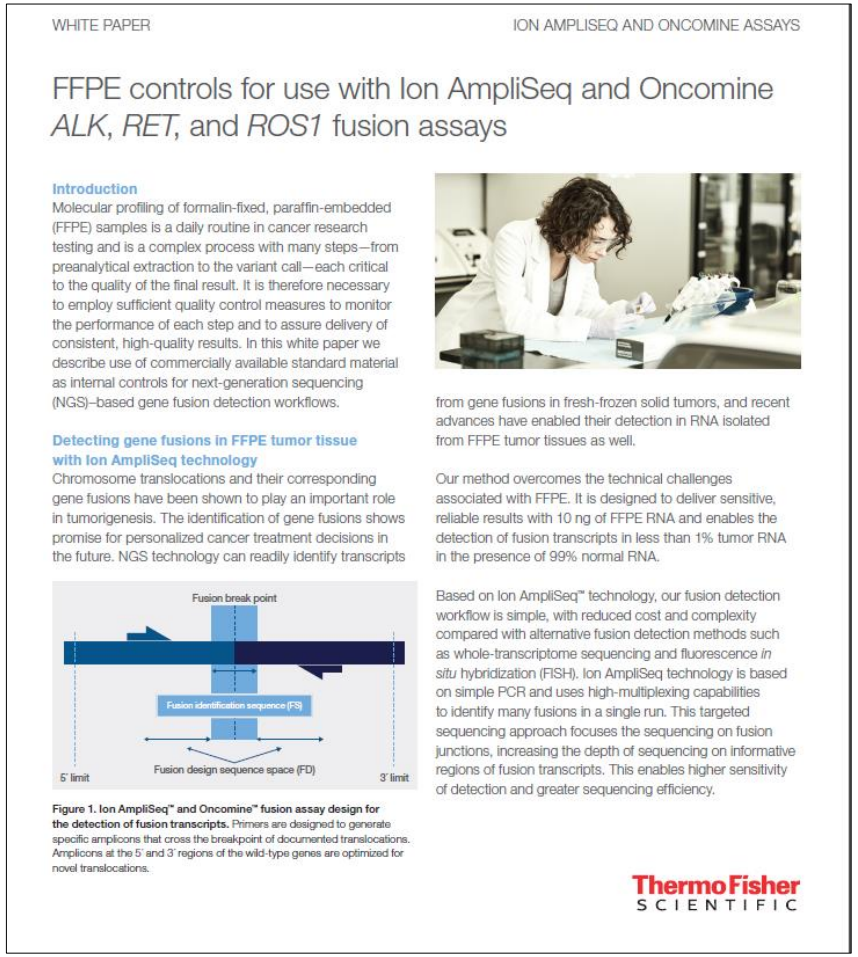
Gene	Variant
Format	1 FFPE curl
Amount of extractable RNA	>100ng
Fusions (and COSMIC IDs)	EML4-ALK (COSF463) CCDC6-RET (COSF1272) SLC34A2-ROS1 (COSF1197) TPM3-NTRK1 (COSF1330) ETV6-NTRK3 (COSF572)
Quality Certificates	ISO 9001
Companion Product	5-Fusion Multiplex Negative Control (HD783)
Catalogue Number	HD796



WHITE PAPER ION AMPLISEQ AND ONCOMINE ASSAYS

FFPE controls for use with Ion AmpliSeq and Oncomine *ALK*, *RET*, and *ROS1* fusion assays

Introduction

Molecular profiling of formalin-fixed, paraffin-embedded (FFPE) samples is a daily routine in cancer research testing and is a complex process with many steps—from preanalytical extraction to the variant call—each critical to the quality of the final result. It is therefore necessary to employ sufficient quality control measures to monitor the performance of each step and to assure delivery of consistent, high-quality results. In this white paper we describe use of commercially available standard material as internal controls for next-generation sequencing (NGS)–based gene fusion detection workflows.

Detecting gene fusions in FFPE tumor tissue with Ion AmpliSeq technology

Chromosome translocations and their corresponding gene fusions have been shown to play an important role in tumorigenesis. The identification of gene fusions shows promise for personalized cancer treatment decisions in the future. NGS technology can readily identify transcripts from gene fusions in fresh-frozen solid tumors, and recent advances have enabled their detection in RNA isolated from FFPE tumor tissues as well.

Our method overcomes the technical challenges associated with FFPE. It is designed to deliver sensitive, reliable results with 10 ng of FFPE RNA and enables the detection of fusion transcripts in less than 1% tumor RNA in the presence of 99% normal RNA.

Based on Ion AmpliSeq™ technology, our fusion detection workflow is simple, with reduced cost and complexity compared with alternative fusion detection methods such as whole-transcriptome sequencing and fluorescence *in situ* hybridization (FISH). Ion AmpliSeq technology is based on simple PCR and uses high-multiplexing capabilities to identify many fusions in a single run. This targeted sequencing approach focuses the sequencing on fusion junctions, increasing the depth of sequencing on informative regions of fusion transcripts. This enables higher sensitivity of detection and greater sequencing efficiency.

Figure 1. Ion AmpliSeq™ and Oncomine™ fusion assay design for the detection of fusion transcripts. Primers are designed to generate specific amplicons that cross the breakpoint of documented translocations. Amplicons at the 5' and 3' regions of the wild-type genes are optimized for novel translocations.

ThermoFisher SCIENTIFIC

- Recommended in 2017 ***Thermo White Paper***
- Suitable for use with the majority of fusion assays
  - Thermo Oncomine Focus
  - Archer FusionPlex assays
  - Illumina TST 170
- Can be used for ***assay development, validation, primer QC and sensitivity assessment***

Feel free to ask any question or speak to our team!

www.horizondiscovery.com/contact-us