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TECHNICAL NOTE

Use of an alternative MIDI plate in the TruSight™ Oncology 500 workflow

Assessment of the VWR Deep Well Plate as a substitute for the Fisher Scientific MIDI plate in library preparation steps

Comparable DNA results

The VWR plate showed similar performance to the Fisher Scientific plate with control DNA and FFPE-derived samples

RNA limitations observed

Reference RNA samples showed reduced coverage and higher QC failure rates with the VWR plate

Lab validation recommended

The VWR plate is not validated by Illumina; labs should verify performance before use

Introduction

Illumina TruSight Oncology 500 is a comprehensive next-generation sequencing (NGS) assay that enables comprehensive genomic profiling (CGP). Due to ongoing shortages of certain plastic consumables, some laboratories have experienced difficulty sourcing the Fisher Scientific* 96-well 0.8 ml MIDI plate (Thermo Fisher Scientific, Catalog no. AB-0859), a consumable specified in the TruSight Oncology 500 Reference Guide.[1] This technical note reviews the use of the VWR† 96-well Deep Well Plate (VWR, Catalog no. 76210-524) as an alternative in the library preparation steps in the TruSight Oncology 500 Research Use Only workflow.

The thermal properties of the Fisher Scientific and VWR plates were compared and differences in library sequence performance were assessed. While the results indicate that the VWR plate may be a viable substitute for the specified use, this substitution has not been validated by Illumina and performance is not guaranteed. It is recommended that laboratories independently evaluate and verify performance before adopting this alternative in their workflows.

Methods

Thermal and functional tests were conducted to evaluate the use of VWR 96-well Deep Well Plates as an alternative to Fisher Scientific 96-well MIDI plates (Table 1) in the Illumina TruSight Oncology 500 workflow. All incubations for thermal and functional testing were performed on the same Hybex Microsample Incubator (SciGene, Catalog no. 1057-30-O) with an Illumina MIDI Heat Block Insert (Illumina, Catalog no. BD-60-601).

* Fisher Scientific is a brand of Thermo Fisher Scientific.
† VWR is a subsidiary of Avantor.

The plates were assessed for differences in heating properties by adding 100 µl nuclease-free dH_2O to five wells per plate at 57°C for five minutes and recording final temperatures with a thermocouple (Extech, Catalog no. TM500). Three replicates of each plate type were assessed.

To evaluate nonspecific DNA binding, 25 ng of fragmented Seraseq reference control DNA (SeraCare, Catalog no. 10592800) in 100 µl Resuspension Buffer (RSB; included in the TruSight Oncology 500 library prep kit) was incubated at 72°C for 20 minutes, and pre- and post-incubation concentrations were measured using the Qubit 1X dsDNA High Sensitivity (HS) Assay Kit (Thermo Fisher Scientific, Catalog no. Q33230).

DNA and RNA libraries were prepared following the TruSight Oncology 500 standard protocol.[1] Libraries were prepared from 40 ng Seraseq reference control DNA or Seraseq reference control RNA (SeraCare, Catalog no. 10597544) and 40 ng DNA or RNA extracted from FFPE samples (Illumina internal samples). For comparative analysis, libraries were prepared in parallel using either the Fisher Scientific plate or the VWR plate, with each plate type used consistently throughout all steps of the protocol that specified the MIDI plate. Library prep was performed manually or with partial automation using the Hamilton Microlab STAR Liquid Handler (Hamilton, Catalog no. HMLT-STAR-B575), with all manual steps executed by the same operator.

Functional runs consisted of sequencing prepared libraries on the Illumina NextSeq™ 550 System and analysis using the TruSight Oncology Comprehensive Analysis Module V2.9.5. Data were evaluated using run-level and library-level QC metrics, including median insert size, usable microsatellite instability (MSI) sites, percent of reads on target (PCT target), and median exon coverage for DNA samples, and median insert size, total on-target reads, and median CV gene 500X for RNA

Table 1: Plate Specifications

Supplier	Product name	Catalog no.	Plastic type	Volume per well	Plate weight	Sterility	Max centrifuge
Thermo Fisher Scientific	Fisher Scientific 96-well MIDI storage plate[a]	AB-0859	Polypropylene	0.8 ml	78.0 g	DNase/ RNase Free	2000 × g
VWR/Avantor	VWR 96-well Deep Well Plate	76210-524	Polypropylene	0.8 ml	77.0 g	DNase/ RNase Free	10,000 × g

a. Current full product name is Abgene 96 Well 0.8mL Polypropylene DeepWell Sample Processing & Storage Plate for Genomics and NGS library preparation.

samples. The FFPE-derived DNA and RNA samples each contained a variant in one of the targeted regions to allow for sensitivity evaluation.

Results

Thermal properties

Thermal measurements revealed that wells in the VWR plates consistently reached 1–2°C lower final temperatures than wells in the Fisher Scientific plates under identical heating conditions. Nonspecific DNA binding tests showed no significant difference between the Fisher Scientific and VWR plates, with comparable DNA recovery following incubation at 72°C.

Control sample performance

Experiments using reference control samples included five functional runs using eight DNA and RNA replicates each. Libraries prepared with VWR plates showed reduced coverage metrics for both DNA and RNA control samples compared to samples prepared with Fisher Scientific plates. While DNA libraries still met all specification limits (Figure 1A), RNA libraries showed reduced coverage, elevated median insert sizes, and an increased rate of functional failures due to shifts in total on-target reads (Figure 1B).

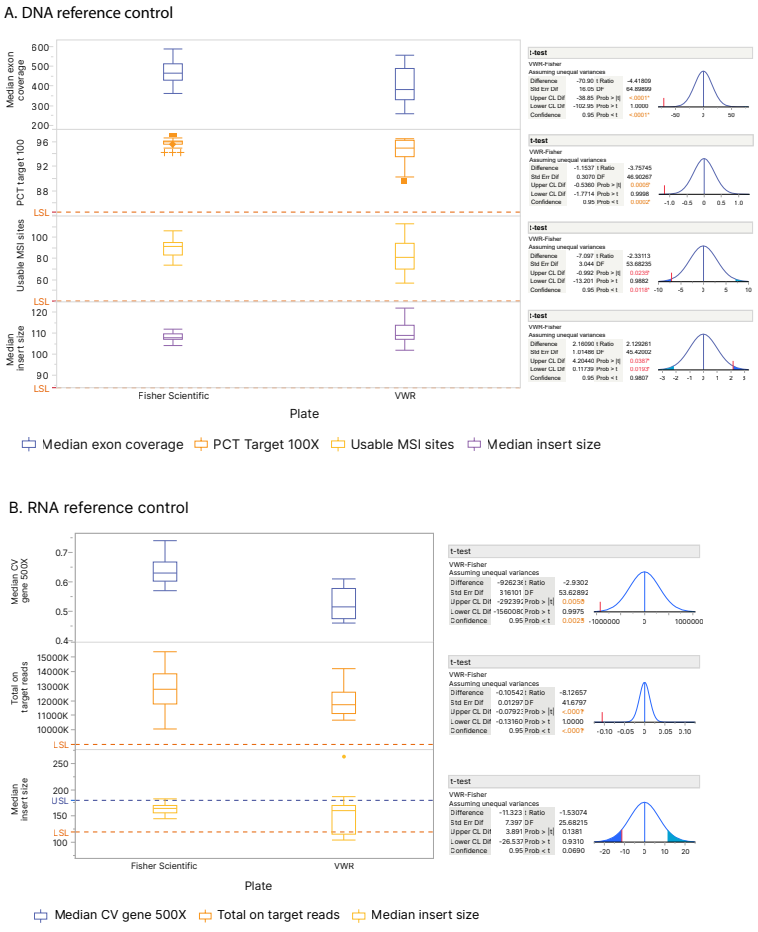

Figure 1: Comparison of reference control DNA and RNA library QC metrics for Fisher Scientific vs VWR Plates

Side-by-side library preparations from reference control (A) DNA and (B) RNA using Fisher Scientific or VWR plates, with eight replicates each. The bar charts on the left indicate the mean sequencing metrics for each plate (± SEM). Histograms on the right depict the null distribution of t-tests under the null hypothesis of no difference between plates. The RNA libraries prepared using the VWR plates showed elevated median insert sizes with an increased rate of functional failures due to shifts in total on-target reads.

CV, coefficient of variation; LSL, lower specification limit; MSI, microsatellite instability; PCT, percent target bases; SEM, standared error of the mean; USL, upper specification limit.

Because the metrics for RNA samples showed the most pronounced impact, an RNA-only run was conducted using pooled cDNA libraries, with half tested using VWR plates and the other half with Fisher Scientific plates. Libraries generated with VWR plates exhibited consistently lower total on-target reads, with a ~3% decrease compared to the number of on-target reads generated with Fisher Scientific plates.

FFPE sample performance

For functional runs using FFPE-derived samples, eight replicates each of DNA and RNA samples were evaluated. The libraries were treated identically except for steps using the MIDI plates, for which half of the samples were processed using a Fisher Scientific plate and the other half were processed using a VWR plate. Libraries prepared from FFPE-derived samples showed equivalent performance between plate types, with no observable differences in library QC metrics (Figure 2). Sensitivity for both DNA and RNA, defined by detection of expected variants, was maintained across all runs, regardless of plate type.

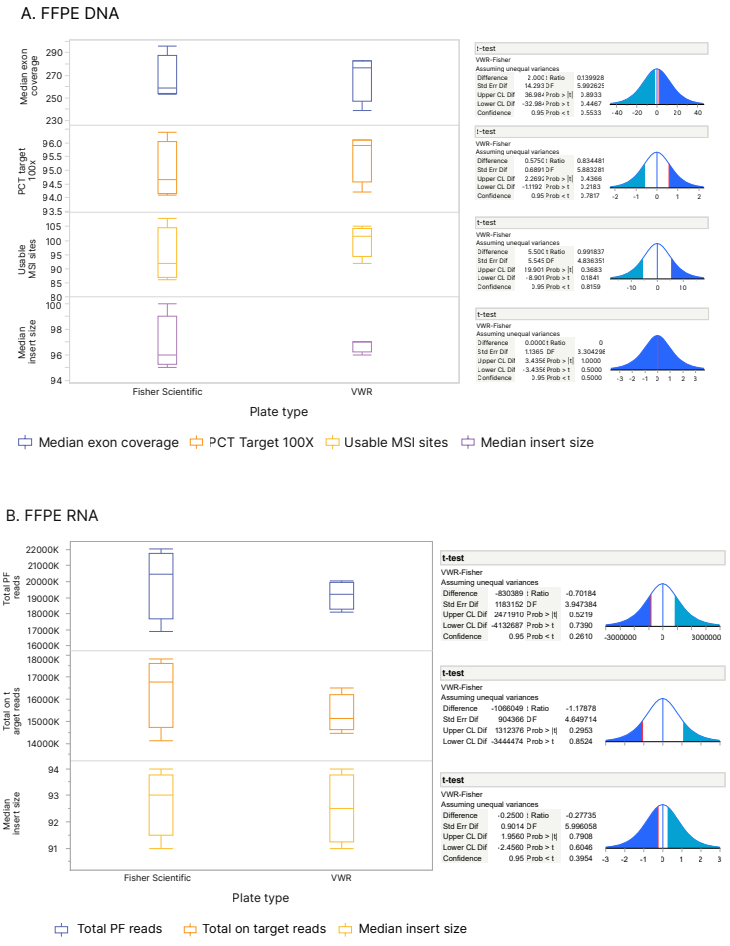

Figure 2: Comparison of FFPE-derived DNA and RNA library QC metrics for Fisher Scientific vs VWR Plates

Side-by-side library preparations from FFPE-derived (A) DNA and (B) RNA using Fisher Scientific or VWR plates, with eight replicates each. The bar charts on the left indicate the mean sequencing metrics for each plate (± SEM). Histograms on the right depict the null distribution of t-tests under the null hypothesis of no difference between plates. No significant differences were observed in QC metrics between plate types for either DNA or RNA libraries.

MSI, microsatellite instability; PCT, percent target bases; PF, passing filter; SEM, standared error of the mean.

Summary

While the VWR 96-well Deep Well Plate performed comparably to the Fisher Scientific 96-well MIDI storage plate for libraries prepared from DNA reference control and FFPE-derived DNA and RNA samples, its use was associated with reduced library coverage and increased failure rates when using RNA reference control samples. The VWR plates have been evaluated solely for the TruSight Oncology 500 Research Use Only workflow. It is recommended that laboratories assess the performance of the VWR plate within their own workflows before implementation.

Reference

1. Illumina. TruSight Oncology 500 Reference Guide support. illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/trusight/oncology-500/1000000067621_11_trusight-oncology-500-reference-guide.pdf. Published January 2023. Accessed May 22, 2025.

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