

MRD Express Datasheet

INTRODUCTION

Twist MRD Express panels are designed for research and characterization of low-frequency variants in cfDNA libraries. This data sheet details the general performance expectations for MRD Express panels with real cfDNA libraries, including input mass, sequencing depth, UMI stringency for a desired mean target coverage, and low % variant allele frequency (VAF) detection.

RESULTS

NATIVE cfDNA PERFORMANCE

Twist routinely uses the Twist cfDNA Pan-Cancer Reference Standard v2 to measure performance of MRD Express panels. Shown below is a comparison of MRD Express capture performance using both Twist cfDNA Pan-Cancer Reference Standard v2 and native cfDNA libraries.

MRD Express capture performance is comparable between native cfDNA and reference standard libraries.

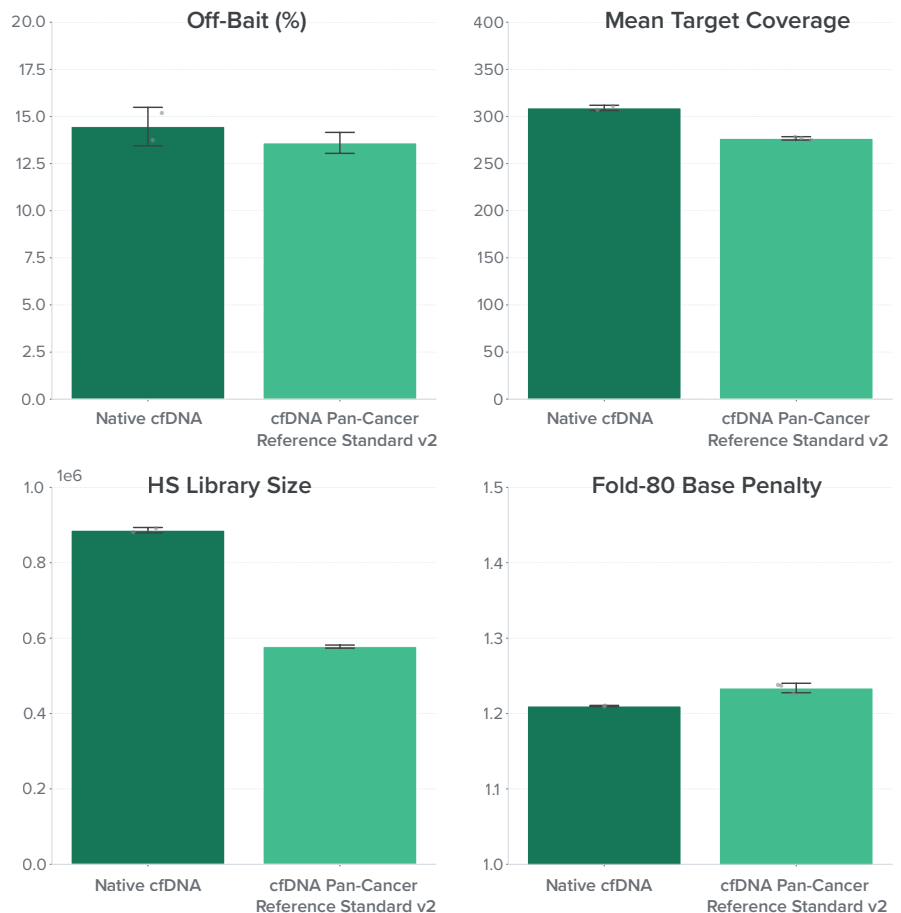


Figure 1. Picard metrics for MRD Express capture of native, commercially sourced cfDNA and Twist cfDNA Pan-Cancer Reference Standard v2. Native cfDNA was extracted originally from EDTA whole blood donation. Plasma was separated before a magnetic bead-based cfDNA extraction. 15 ng of native cfDNA or cfDNA Pan-Cancer Reference Standard v2 was used in Twist's cfDNA Library Preparation Kit with UMI adapters. 500 ng of each library was used in Twist's Standard Hybridization Reagent Kit v2 and sequenced on a NextSeq 500 2x75, and aligned for Picard metrics after a 1,000x global downsample.

DON'T SETTLE FOR LESS IN TARGETED SEQUENCING.

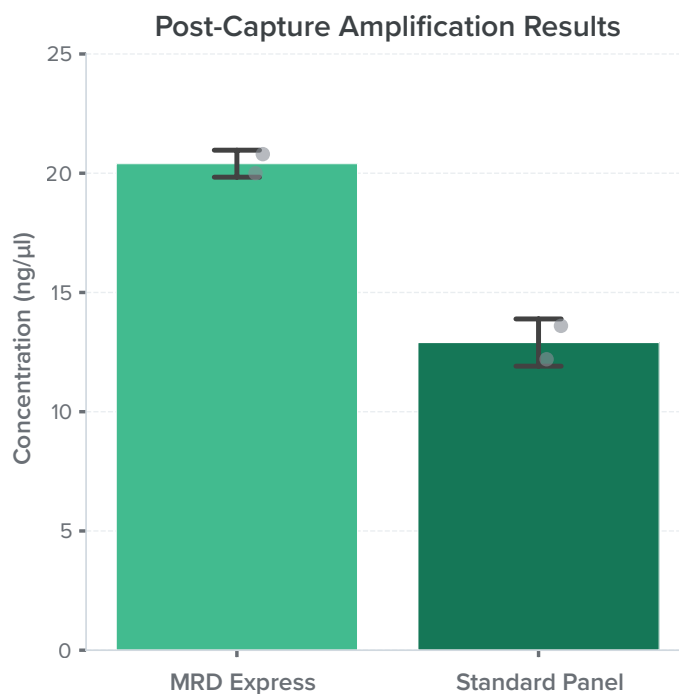
Get in touch at sales@twistbioscience.com or learn more at twistbioscience.com/products/ngs



POST-CAPTURE YIELD

An important consideration for capture probe selection is the generation of enough mass to sequence after post-capture PCR amplification. MRD Express panels give equivalent or higher yield than matched standard panels.

Figure 2. Concentration in ng/μl comparing MRD Express and standard panels. 15 ng of Native cfDNA or cfDNA Pan-Cancer Reference Standard v2 was used in Twist's cfDNA Library Preparation Kit with UMI adapters. 500 ng of each library was used in Twist's Standard Hybridization Reagent Kit v2 with a matched 400-target MRD Express or 400-target standard panel. Twist TrueAmp Polymerase Mix with 18 PCR cycles were used for both captures. Concentration was assayed with a Qubit dsDNA Quantification Assay Kit.



SEQUENCE DEPTH REQUIREMENTS

Unique coverage is the primary factor in determining how sensitive a sequencing assay is towards rare variants. For example, in an experiment that profiles a 0.1% VAF sample with a mean coverage of 1,000x, this would translate to approximately 1 detection per site on average. Mean target coverage for a capture library is dictated by four factors: cfDNA input mass, sequencing depth, UMI collapse stringency, and capture performance.

1. Higher mass inputs result in a more complex library with more unique molecules available for capture. Note that the chart below uses the high-conversion Twist cfDNA Library Preparation kit, and other library preparation kits may have lower conversion, leading to lower library complexity and mean target coverage.
2. Sequencing depth refers to how many reads are given to a captured library. The higher the sequencing depth, the more unique molecules are sequenced, which in turn increases mean target coverage. Higher sequencing depth will eventually hit a point of diminishing returns, where most reads detect existing sequences rather than detecting additional unique molecules.
3. UMI collapse stringency affects mean target coverage since more stringent cutoffs for UMI family size means that families with less support are discarded. For example, in a 6-3-3 filter, all families with less than 6 total reads, or less than 3 reads on each strand, would be filtered out as having insufficient error correction power. Using a lower-stringency filter will enable detection of more unique molecules, at the expense of potentially less accurate base calls. Refer to "[https://www.twistbioscience.com/resources/guideguideline/twist-umi-design-overview-and-usage-guidelines](\"https://www.twistbioscience.com/resources/guideguideline/twist-umi-design-overview-and-usage-guidelines\")" for more information.
4. Capture performance is the critical final step to enable high mean target coverage. Low off-bait with high complexity and uniformity are vital. MRD Express panels are compatible with Twist's hybridization reagents.

The chart below illustrates the impact of input mass, sequencing depth, and UMI collapse when using Twist library preparation and hybridization reagents.



cfDNA INPUT MASS (ng)	SEQUENCING DEPTH (1000X)	MEAN TARGET COVERAGE						
		1-0-0	2-0-0	3-0-0	2-1-1	3-1-1	4-2-2	6-3-3
1	5	230 ± 10	210 ± 10	200 ± 10	125 ± 5	124 ± 5	105 ± 4	82 ± 3
5	5	790 ± 10	480 ± 40	260 ± 40	210 ± 20	150 ± 20	50 ± 10	9 ± 4
15	5	1210 ± 20	409 ± 1	119 ± 2	159 ± 2	71.5 ± 0.6	10.8 ± 0.2	0
30	5	1450 ± 20	253 ± 4	38 ± 2	87 ± 1	21.7 ± 0.4	1.6 ± 0.2	0
1	10	240 ± 10	230 ± 10	220 ± 10	138 ± 6	138 ± 6	131 ± 5	120 ± 5
5	10	996 ± 8	800 ± 20	600 ± 40	400 ± 10	360 ± 20	190 ± 30	70 ± 20
15	10	1810 ± 30	984 ± 8	474.3 ± 0.8	431 ± 9	290 ± 3	81.0 ± 0.7	10.5 ± 0.6
30	10	2480 ± 30	770 ± 10	208 ± 6	287 ± 4	120 ± 2	16.4 ± 0.3	0
1	20	240 ± 20	240 ± 10	230 ± 10	143 ± 6	143 ± 6	141 ± 6	137 ± 6
5	20	1130 ± 20	1039 ± 9	926 ± 8	590 ± 10	571 ± 9	430 ± 10	280 ± 30
15	20	2390 ± 40	1790 ± 20	1240 ± 10	910 ± 30	790 ± 20	369 ± 4	116 ± 2
30	20	3810 ± 50	1920 ± 20	858 ± 9	805 ± 9	512 ± 5	128 ± 1	14.43 ± 0.03
1	40	250 ± 20	240 ± 10	240 ± 10	145 ± 6	144 ± 6	144 ± 6	143 ± 6
5	40	1210 ± 40	1160 ± 30	1120 ± 20	690 ± 40	690 ± 40	620 ± 20	530.1 ± 0.8
15	40	2790 ± 50	2490 ± 40	2140 ± 30	1450 ± 60	1390 ± 50	960 ± 20	548 ± 7
30	40	5140 ± 70	3670 ± 40	2410 ± 20	1780 ± 30	1480 ± 20	635 ± 6	176.6 ± 0.7
1	80	260 ± 20	240 ± 20	240 ± 20	146 ± 6	145 ± 6	145 ± 6	144 ± 6
5	80	1240 ± 40	1210 ± 40	1200 ± 30	730 ± 50	730 ± 50	710 ± 40	680 ± 30
15	80	2990 ± 20	2840 ± 20	2650 ± 50	1770 ± 20	1750 ± 10	1460 ± 60	1100 ± 100
30	80	6130 ± 90	5320 ± 70	4440 ± 50	2980 ± 60	2820 ± 50	1820 ± 20	956 ± 7
15	160	3087	3020	2962	1906	1905	1829	1700
30	160	6628	6273	5852	3815	3777	3140	2385

Table 1. 1-30 ng of cfDNA Pan-Cancer Reference Standard v2 was used in Twist's cfDNA Library Preparation Kit with UMI adapters and the Twist cfDNA Target Enrichment Standard Hybridization workflow. Samples were sequenced on a NextSeq 2000 and aligned for Picard metrics after fgbio UMI filtering.

LOW % VAF DETECTION

To ensure MRD Express panels could detect VAFs as low as 0.001% or 10 Parts Per Million (PPM), libraries were generated with 15 ng serially diluted Twist cfDNA Pan-Cancer Reference Standard v2 1% VAF. Each %VAF library was then captured (500 ng each) using MRD Express panels and Twist Standard Hybridization Reagent Kit v2. The libraries were also captured with standard Twist custom panels. Post-capture libraries were sequenced on a NovaSeq 10B flow cell and downsampled to 80,000x coverage per library.

For the 1% VAF standard, nearly all sites were detected using a 2-1-1 UMI collapse. At 0.1% VAF about 80% of sites were detected. At 0.001% VAF, about 4% of sites were detected. Using a one-sided exact permutation test, the 0.001% VAF or 10PPM condition gives statistically significant total recall when compared to the background in the 0% VAF sample ($p=0.001$).

This example illustrates how input mass, sequencing depth, UMI collapse stringency, and capture performance integrate to enable %VAF calls at low levels and shows that MRD Express panels perform equivalently to standard Twist panels.

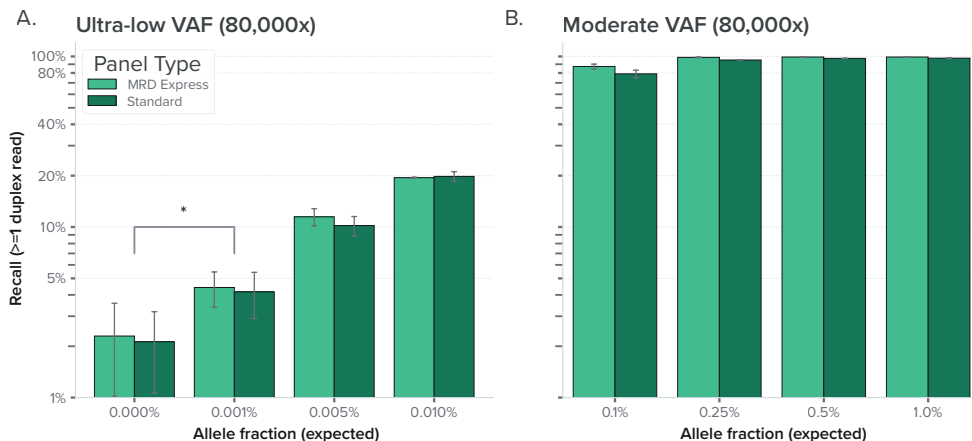


Figure 3. 15 ng of cfDNA Pan-Cancer Reference Standard v2 was used in Twist's cfDNA Library Preparation Kit with UMI adapters and Twist's Standard Hybridization Reagent Kit v2 with a 400-target MRD Express panel or matched 400-target standard panel. Samples were sequenced on a NextSeq 550 and aligned for Picard metrics after fgbio UMI filtering. Overall recall among all sites is shown for both MRD Express panels and standard panels.

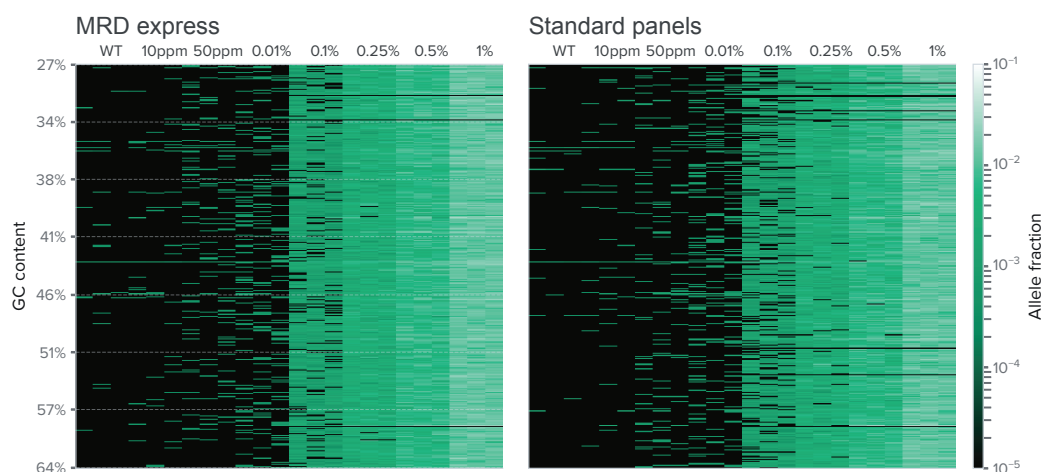


Figure 4. 15 ng of cfDNA Pan-Cancer Reference Standard v2 was used in Twist's cfDNA Library Preparation Kit with UMI adapters and Twist's Standard Hybridization Reagent Kit v2 with a 400-target MRD Express panel or matched 400-target standard panel. Samples were sequenced on a NextSeq 550 and aligned for Picard metrics after fgbio UMI filtering. Recall allele fraction is shown across different VAF libraries for both MRD Express panels and standard panels.

PANEL INFORMATION

ATTRIBUTE	DETAIL
Panel size	1 to 10,000 probes
Variant types	SNVs, small indels, CNVs, and exonic target regions
Design input	Variant target coordinate BED file or FASTA probe sequences
Genome builds	hg19 and hg38
Workflow compatibility	Existing Twist library preparation and hybrid capture workflows
Recommended protocol	Twist Standard Hybridization v2 and Fast Hybridization
Ordering	Twist eCommerce platform or API integration for LIMS-connected ordering
Shipping Tubes	0.5-ml Sarstedt, FluidX or Matrix (available through API)
Intended use	Research Use Only

Scan the QR code to learn more.



Design and order your panel.



Turnaround times are estimates only and are not guaranteed. Actual turnaround may vary based on order specifications, sample characteristics, processing requirements, operational capacity, and other applicable factors. Product information, specifications, features, availability, and timing are subject to change without notice. Twist products are for research use only. They are not intended for the diagnosis, prevention, monitoring, or treatment of a disease or condition. Twist Bioscience assumes no liability regarding the use of its products in unintended applications.