xGen™ MRD Hybridization Panel for Minimal Residual Disease Research

A complete sample preparation workflow solution for variant identification of circulating tumor DNA (ctDNA)

Panels delivered within 5 business days from placing your order

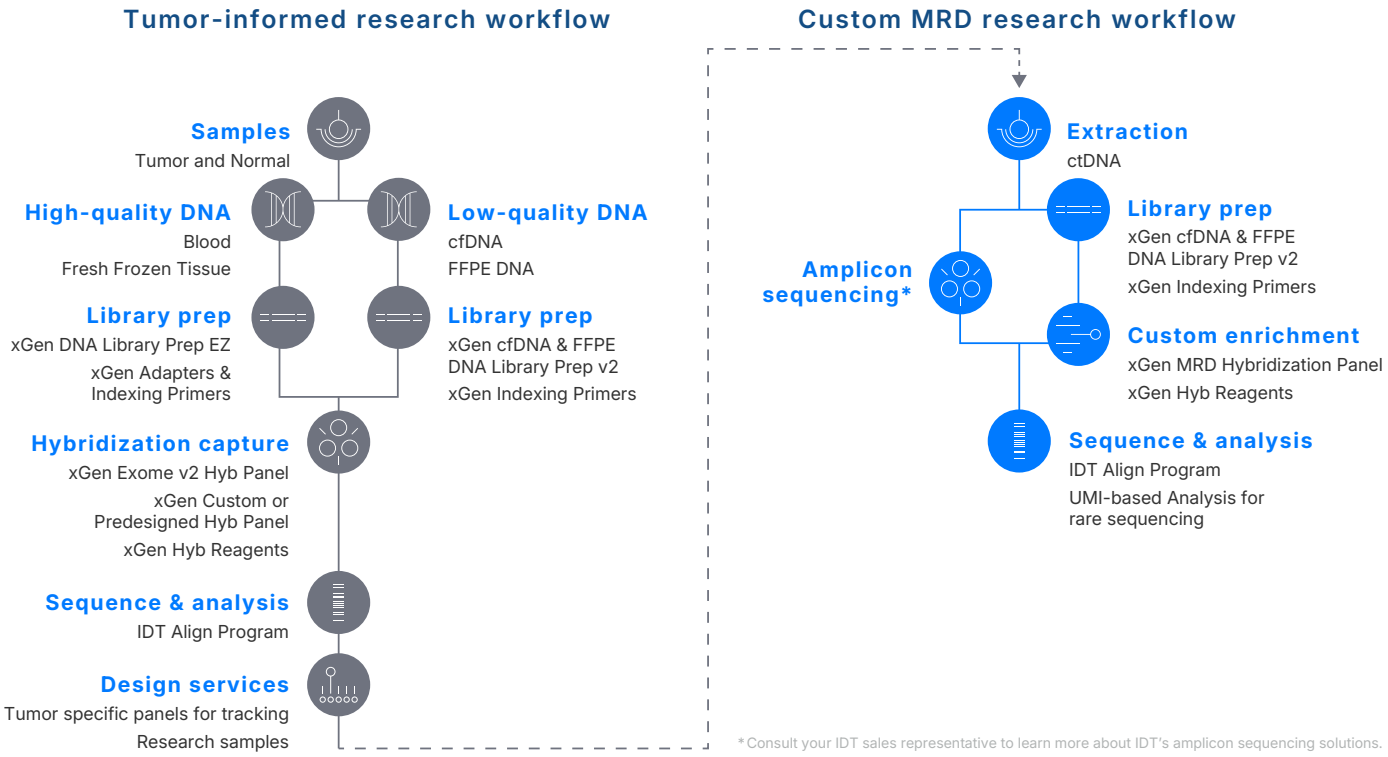
Order up to 50 panels at once through the MRD web ordering tool

A custom solution for under $40 per sample

MRD workflow overview

Minimal residual disease (MRD) refers to the presence of residual cancer cells which remain in the body at low levels [1]. MRD research using NGS requires a full solution encompassing initial variant discovery from individual tumors, followed by custom target enrichment of those variants from low-abundance cell-free DNA (cfDNA) in identifying the presence of circulating tumor DNA (ctDNA) which is indicative of MRD.

For rare variant discovery, IDT offers an xGen Custom MRD Workflow to assist you in your MRD research needs. Easily prepare high complexity libraries from extracted cfDNA using the **xGen cfDNA & FFPE DNA Library Prep Kit**. For target enrichment and ultra-low variant identification, design your xGen MRD Hybridization Panels with up to 2000 probes per panel and order up to 50 panels at once. These customizable panels enable reliable variant identification at ≤1% variant allele frequency (VAF) if input cfDNA quantity and sequencing depth requirements are met (**Table 1**).



*Consult your IDT sales representative to learn more about IDT's amplicon sequencing solutions.

RUO For Research Use Only. Not for use in diagnostic procedures.

INTEGRATED DNA TECHNOLOGIES

Overcoming MRD research challenges

Identifying ultra-low frequency variants in research samples is a challenge experienced when studying MRD. IDT offers a tumor-informed workflow that starts with a custom or predesigned panel that has a broad target region to identify variants that are representative of each specific cancer (**Table 1**). Our panels are designed to reduce off-target binding while maximizing coverage for identifying variants in research samples. The identified variants then form the basis for a customized tumor-specific **xGen MRD Hybridization Panel**. The recurrent use of this panel with deep sequencing can then be used to track the presence or absence of specific biomarkers at ultra-low frequency.

The unique, single-stranded ligation strategy of the IDT **xGen cfDNA & FFPE DNA Library Prep v2 MC Kit** and workflow delivers high conversion of input DNA molecules to sequencing data. This high conversion rate is critical for identification of ultra-low frequency variants—common in the analysis of cfDNA samples. A higher conversion rate translates to higher complexity and coverage than other DNA library prep kits for cfDNA (**Figure 1**). Combining higher complexity and coverage with stringent error correction better enables the identification of ultra-low frequency variants (**Table 1**).

Table 1. xGen cfDNA & FFPE DNA Library Prep Kit identifies low frequency variants in NGS reference samples.

Mutation	Expected VAF	xGen cfDNA & FFPE DNA Library Prep v2 MC Kit*	Library Kit A*	Library Kit B*
EGFR: L858R	0.25%	0.13 (3/3)	0.21 (3/3)	0.21 (3/3)
EGFR: E746-A750	0.25%	0.11 (3/3)	0.19 (3/3)	0.12 (3/3)
EGFR: T790M	0.25%	0.29 (3/3)	0.36 (3/3)	0.12 (3/3)
KRAS: G12D	0.32%	0.33 (3/3)	0.36 (3/3)	0.33 (3/3)
NRAS: Q61K	0.32%	0.23 (3/3)	0.31 (2/3)	0.22 (3/3)
NRAS: A59T	0.32%	0.17 (3/3)	0.43 (2/3)	0.22 (3/3)
PIK3CA: E545K	0.32%	0.16 (3/3)	0.11 (3/3)	0.36 (3/3)

*Libraries were prepared in triplicate from 50 ng input Horizon cfDNA reference standards using the xGen cfDNA & FFPE DNA Library Prep v2 MC Kit in addition to two other commercially available library prep kits. Libraries were then captured with a 180 kb (target space) xGen Custom Hyb Panel targeting seven identified SNVs using the using the xGen Hybridization and Wash Reagents and Beads v2. Captured libraries were pooled and sequenced on a NextSeq™ 500 (Illumina), using a high output 300 cycle kit and the manufacturer's protocol. After subsampling to 85M total reads, the average variant allele frequency (VAF) for each of the targeted mutations was calculated by library prep kit using VarDict.

Key benefits—xGen solutions for MRD research

- **More complete workflow**—curated workflow for custom MRD hybridization panels
- **Turnaround time**—proven TAT of 5 business days from order being placed
- **Flexible panel size**—target tumor-specific variants with up to 2000 probes per panel
- **Affordable MRD research**—xGen MRD Hybridization Panel delivers a custom solution for <$40 USD per sample
- **Rare variants**—identify variants at ≤1% variant allele frequency (VAF) with your custom MRD panels
- **Automation friendly**—easy workflow that can be automated for high-throughput applications
- **PCR analysis options**—oPools™ Oligo Pools accommodate multiplexed PCR workflows for MRD research

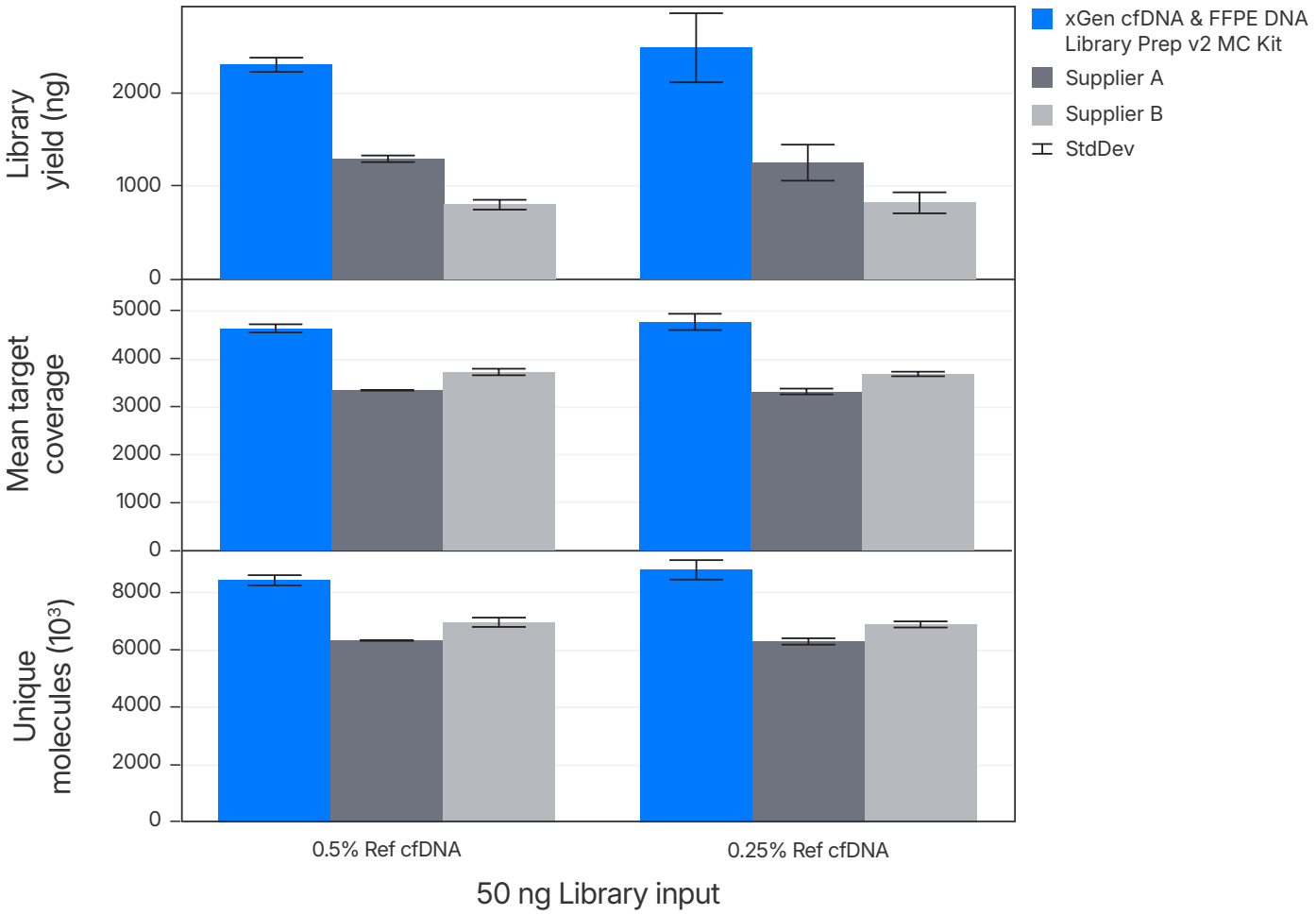


Figure 1. xGen cfDNA &FFPE DNA Library Prep v2 MC Kit delivers higher yields, complexity, and coverage. Libraries were prepared in triplicate according to the manufacturer's instructions with 50 ng of Horizon cfDNA reference standard and 7 cycles of PCR. Following quantification, libraries were captured with a custom 61 kb (target space) xGen Custom Hyb Panel using the xGen Hybridization and Wash Kit. Captured libraries were pooled and sequenced on a NextSeq™ 500 (Illumina) using a high output 300 cycle kit and the manufacturer's protocol. After subsampling to 85M total reads, coverage and complexity were calculated.

Simple 5 step ordering process

1	Design panel using the xGen Hyb Panel Design Tool **Note:** (Optional) Ask for help from our NGS Application Support team (**www.idtdna.com/ContactUs**).
2	Download the ordering template (up to 50 panels, 2000 probes each)
3	Upload template to the xGen MRD Ordering Tool
4	Panels are added to your cart when the template upload has completed
5	Add any ancillary workflow reagents (RUO) that you may need to your cart and place your order* * Ships within 5 business days after order is placed

Ordering information

Product name	Sizes available
xGen cfDNA & FFPE DNA Library Prep Kit v2 MC Kit	16 & 96 rxn
xGen Hybridization and Wash v2 Reagents	16 & 96 rxn
xGen Hybridization and Wash v2 Beads	16 & 96 rxn
xGen Library Amplification Primer Mix	16 & 96 rxn
xGen Universal Blockers TS	16 & 96 rxn
xGen UDI Primers	16 rxn & Plate 1, 8nt
xGen MRD Hybridization Panel	16 rxn
xGen Normalase™ Module	*96 rxn*

References

1. Thierry AR, El Messaoudi S, Gahan PB, et al. **Origins, structures, and functions of circulating DNA in oncology**. Cancer Metastasis Rev. 2016;35(3):347-376. doi:10.1007/s10555-016-9629-x

For more information, visit **idtdna.com/NGS**