Novel and user-friendly hybridization capture workflow for low quality samples on small gene panels

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Introduction

Hybridization capture targeted sequencing is a valuable and cost-effective method for in-depth sequencing of specific areas of interest to detect desired low allele frequency variants. Integrated DNA Technologies (IDT) novel xGen™ Hyb and Wash v3 (xGen v3) workflow overcomes the laboratory challenges of hybridization capture. The unique hybridization and capture technology eliminates hot liquid handling while demonstrating excellent performance with input and hybridization time flexibility. In this study we demonstrate xGen v3 workflow performance with non-GIAB Formalin-Fixed Paraffin-Embedded (FFPE) input samples using panels of varying sizes from 11 Mb target bed size to custom panel smaller than 200 Kb with different probe design strategies.

Methods

We evaluated xGen v3 workflow performance with non-GIAB Formalin-Fixed Paraffin-Embedded (FFPE) input samples using IDT's stocked and custom panels of varying sizes from 11 Mb target bed size to custom panel smaller than 0.5 Kb with different probe design strategies. Libraries were constructed from Horizon Discovery FFPE standards (pre-extracted or extracted in-house) and NA12878 using IDT's xGen cfDNA & FFPE DNA Library Prep Kit. This was followed by 1-plex hybridization capture using xGen v3 kit workflow with library total input into capture from 500 ng to 100 ng and hybridization times from overnight (ON) to 1 hour. Post capture libraries were sequenced on Illumina®'s NextSeq2000 sequencer. Variant calling was performed using consensus sequencing with VarDict 1.8.3.

Simpler and user-friendly new workflow

Commercially available hybridization capture workflows are notoriously complex and tend to have a long hands-on time and challenging hot liquid washes. xGen v3 has no pre-heating, no hot-liquid handling, and no temperature-sensitive urgency. This workflow significantly reduces the amount of time users must spend at their lab bench while executing the protocol. The simple and user-friendly workflow increases throughput, decreases hands-on time and is easily adaptable for automation.

	xGen v2	xGen v3
Wash buffers to mix/dilute	6	1
Capture and wash hands-on steps	17	9
Hot buffer handling steps	7	0
Hot buffers to pre-heat	3	0
Post-capture sample washes	6	3
Lab & user-dependent: estimation for 32 samples manual processing (min)	117	82

Table1. Comparison of Current IDT xGen Hyb and Wash v2 (xGen v2) workflow and the new xGen v3 workflow. xGen v3 workflow has less buffer mixing, less incubations and no hot buffer pipetting. The simple and user-friendly workflow increases throughput and decreases hands-on time.

High variant calling concordance and sensitivity regardless of hybridization time

All verified SNVs in panel target space were detected across hybridization times and sample input quality
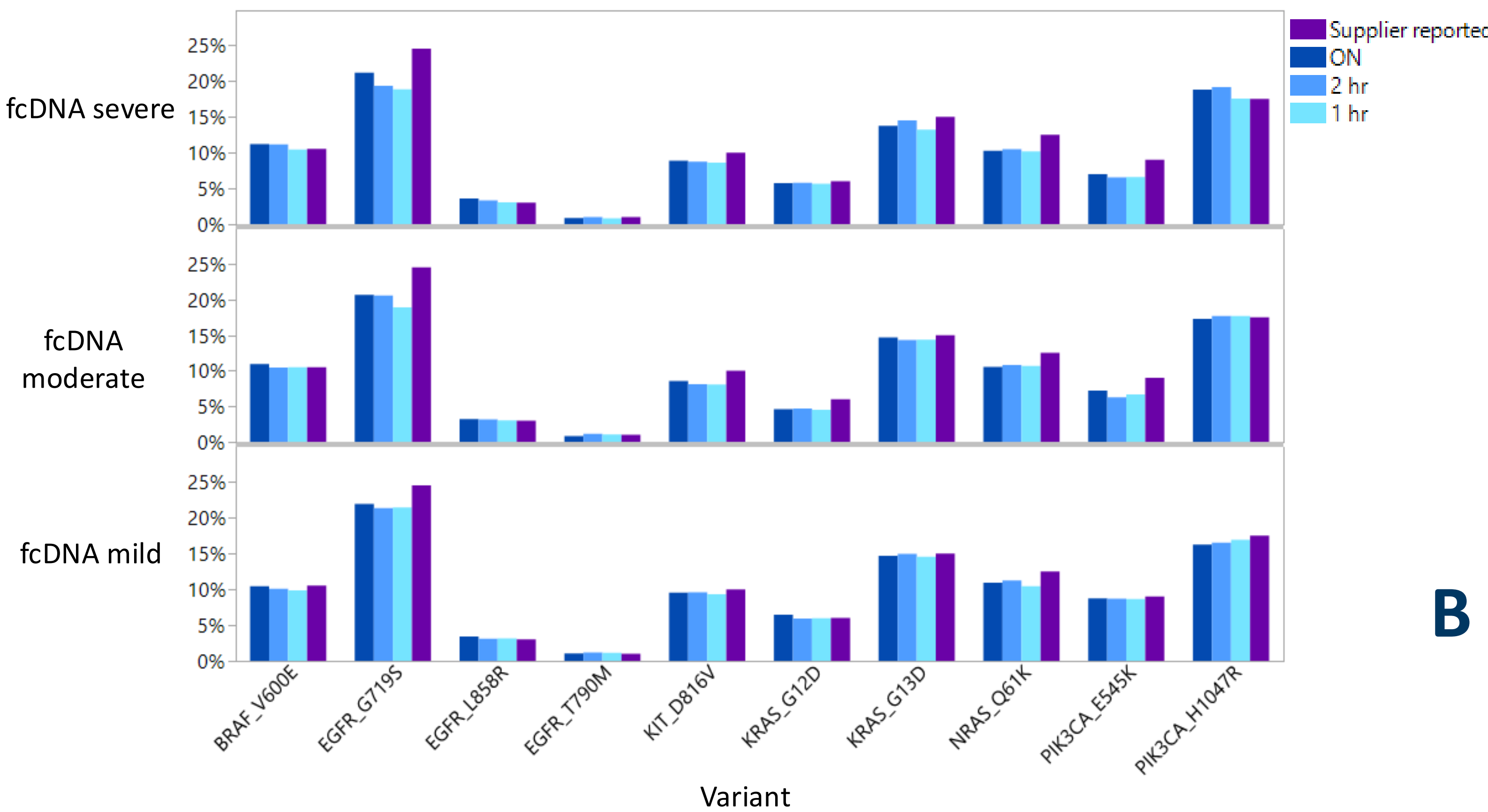

Figure 1. xGen v3 somatic mutation detection across different quality samples. Libraries were constructed from 100 ng Horizon Discovery severe/moderate/mild formalin compromised standard (HD803, HD799 and HD798) using IDT's xGen cfDNA & FFPE DNA Library Prep Kit. This was followed by 1-plex hybridization capture using xGen v3 kit workflow with 500 ng library input into capture. Overnight, 2 hr and 1 hr hybridization with 4 replicates using a custom 180 kb target space IDT panel, sequenced on Illumina® NextSeq2000. Variants were determined with consensus sequencing using Vardict.

IDT custom panel design for Horizon OncoSpan standard variants

Design	Target Bed Size (kb)	Probe Bed Size (kb)
Single strand	0.386	45.48
Double strand (4 base staggered)	0.386	46.9

Table 2. Small custom IDT panels, same OncoSpan Horizon discovery variants targets with different design strategies.

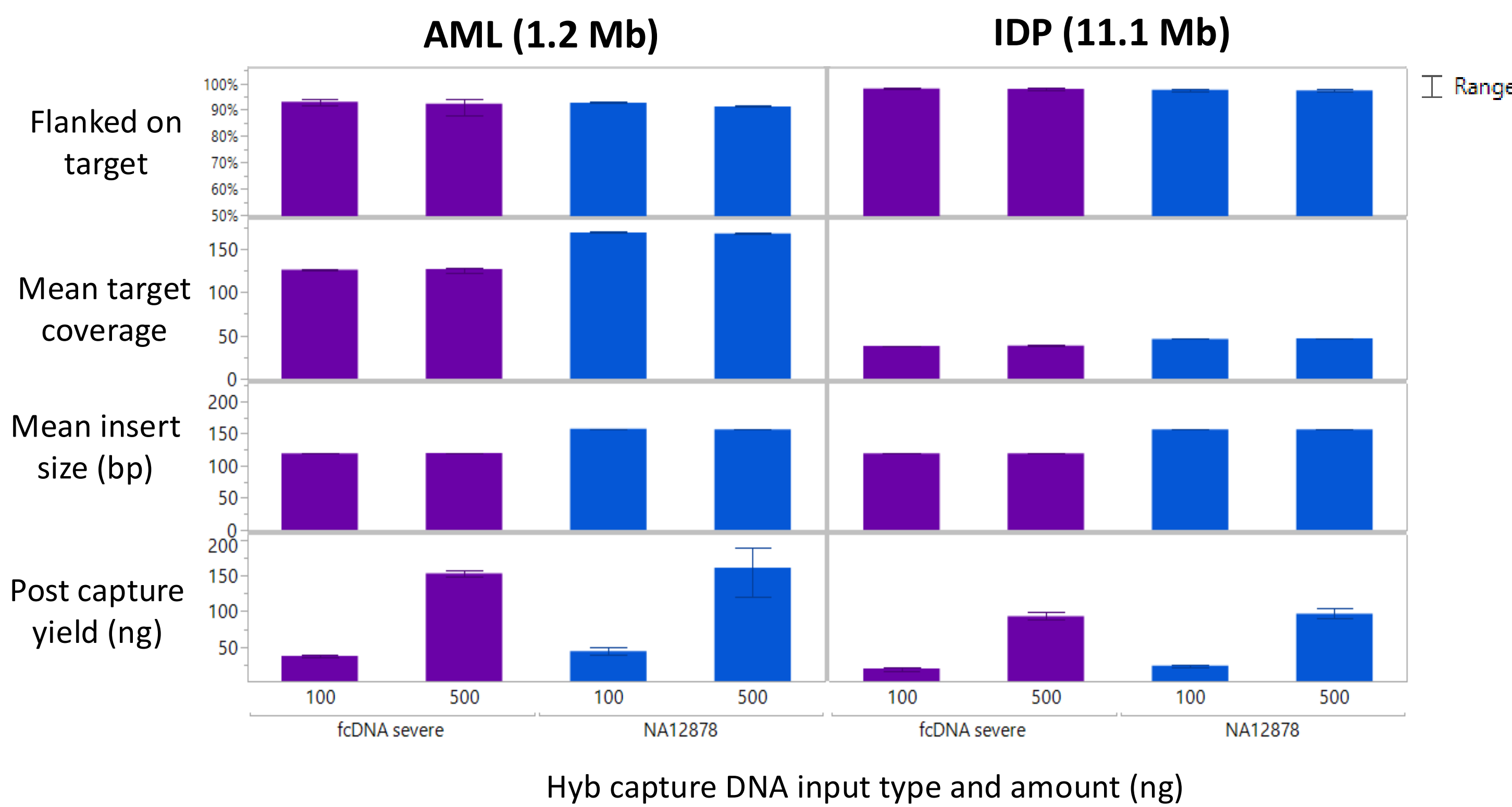## Robust performance with as low as 100 ng input

Figure 2. xGen v3 workflow performance remains high with low input into capture and poor sample quality. Libraries were constructed from 5ng Horizon Discovery severe formalin compromised standard (HD803) and NA12878 using IDT's xGen cfDNA & FFPE DNA Library Prep Kit. This was followed by 1-plex hybridization capture using xGen v3 kit workflow. Library total input into capture ranged from 500 ng to 100 ng captured using IDT's stocked panels - AML (IDT xGen AML Cancer Hyb Panel) with 12 post capture PCR cycles for both inputs and IDP (IDT xGen Inherited Diseases Hyb Panel) using 10 post capture PCR cycles per input. Each condition contains 4 technical replicates hybridized overnight, sequenced on Illumina® NextSeq2000. IDP was subsampled to 10 M reads and AML to 5 M per sample.

High variant calling concordance and sensitivity regardless of hybridization time

A. Confident variant calling with flexible hybridization time

Panel design	Hybridization time	SNV + InDel (>0.01 AF)	InDel (>0.01 AF)	SNV (>0.01 AF)	AF<=0.05	0.05 <AF <=0.1	0.1<AF<=0.25	0.25<AF<=0.5	0.5<AF<=1
				Sensitivity (observed/expected)					
Single stranded probes	ON	99.5% (373/375)	100% (28/28)	99.4% (345/347)	100% (2/2)	100% (6/6)	100% (59/59)	99.2% (244/246)	100% (34/34)
	2hr	99.5% (373/375)	100% (28/28)	99.4% (345/347)	100% (2/2)	100% (6/6)	100% (59/59)	99.2% (244/246)	100% (34/34)
	1hr	99.5% (373/375)	100% (28/28)	99.4% (345/347)	100% (2/2)	100% (6/6)	100% (59/59)	99.2% (244/246)	100% (34/34)
Double stranded probes	ON	100% (375/375)	100% (28/28)	100% (347/347)	100% (2/2)	100% (6/6)	100% (59/59)	100% (246/246)	100% (34/34)
	2hr	100% (375/375)	100% (28/28)	100% (347/347)	100% (2/2)	100% (6/6)	100% (59/59)	100% (246/246)	100% (34/34)
	1hr	100% (375/375)	100% (28/28)	100% (347/347)	100% (2/2)	100% (6/6)	100% (59/59)	100% (246/246)	100% (34/34)

B. High variants allele frequency concordance between hybridization times and panel probe designs

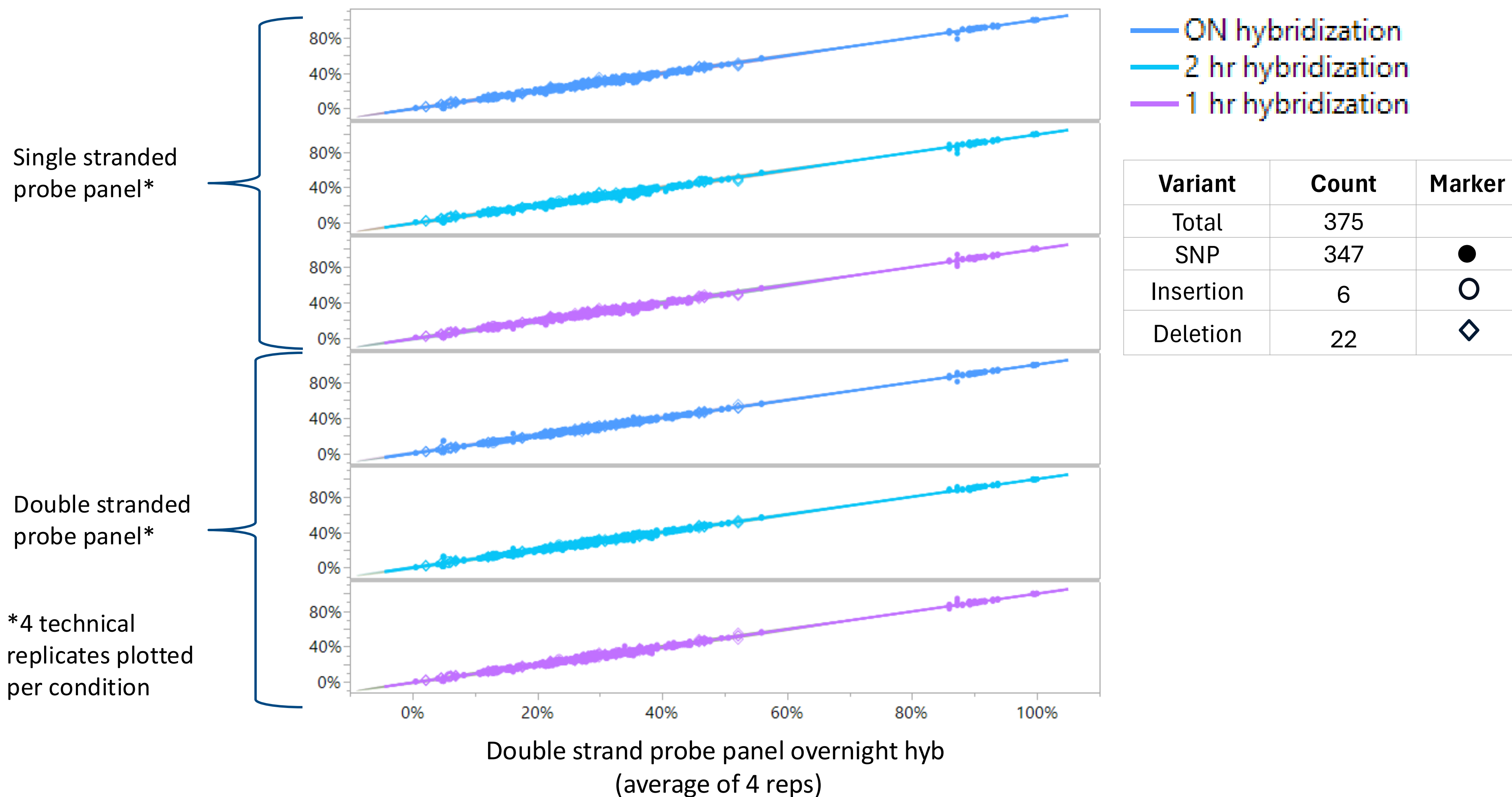| Variant	Count	Marker
Total	375	
SNP	347	●
Insertion	6	○
Deletion	22	◇

Figure 3. xGen v3 high variant calling detection sensitivity and allele frequency concordance with flexible hybridization times and panel design. Libraries were constructed from 100 ng Horizon Discovery OncoSpan FFPE standard (HD832) using IDT's xGen cfDNA & FFPE DNA Library Prep Kit. This was followed by 1-plex hybridization capture using xGen v3 kit workflow with 500 ng library input into capture. Overnight (ON), 2hr and 1hr hybridization with 4 replicates using IDT custom panels (**Table 2**), sequenced on Illumina® NextSeq2000. SNVs and InDels were detected with consensus sequencing using VarDict 1.8.3 for all conditions (**A**). All tested conditions show high variants allele frequency concordance for all replicates (**B**).

Conclusions

- IDT's new xGen Hybridization and Wash v3 Kit:
    - Simpler workflow with significantly lower hands-on time and no hot buffer handling
    - Flexible workflow allowing adjustment of hybridization time to suit needs
    - Generates high on target with as low as 100 ng library input into capture
- Demonstrated good sensitivity to SNVs and InDels detection in challenging FFPE samples
- High concordance in allele frequency with as low as 1 hr hybridization time
- Double-stranded and single-stranded probe panels result in equal sensitivity and variant concordance

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