New streamlined hybridization-capture enables flexible inputs and hybridization times without hot liquid handling

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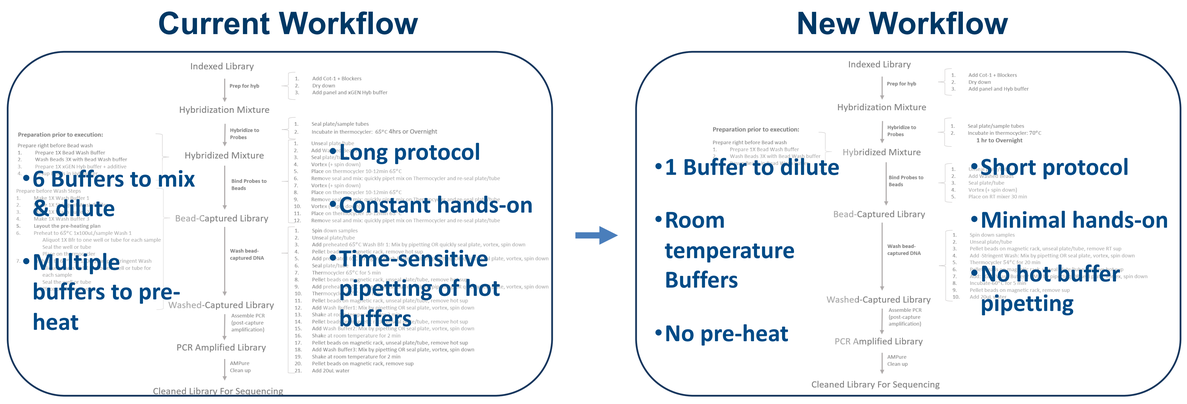
Targeted sequencing by hybridization-capture is a versatile and powerful way to interrogate selected regions of interest in large and complex genomes. However, the laboratory workflow for this approach is notoriously complex and lengthy for commercially available methods.

Here we present a new hybridization-capture workflow that eliminates the laboratory pain points. Benchmarked to commercial Hyb and Wash kit, the number of hands-on steps and reagents are reduced by more than half. The new workflow has no pre-heating, no hot-liquid handling, and no temperature-sensitive urgency. The simple user-friendly workflow increases throughput, decreases hands-on time and is configured for easy automation adaptation. The design and formulations enable equivalent targeting and coverage performance for 1-hour compared to overnight hybridization times with as little as 100ng total library input per hybridization-capture.

The flexibility in hybridization time, library input, and easy workflow makes this hybridization-capture adaptable to a large variety of operational and application needs, from quick surveys to high-throughput and/or in-depth investigations.

Simpler, easier, faster, fewer reagents, fewer steps:



Capture and Wash	Current	New
Buffers to Mix	8	1*
Hands-on Steps	17	8
Hot Handling performed on Thermocycler	7	0
Time-sensitive hot sample handling step	7	0
Pre-Heat Hot buffers	3	0
Incubations	6	3
Total incubation (min)	61	55
No-walk away (min)	61	5
Lab & user-dependent: Estimation for **32** samples **Manual Processing (min)**	117	82

* One buffer supplied at 2X

Red font indicates Pain Point

Figure 1: Comparison of New vs. Current IDT xGen™ Hybridization-Capture reagents and workflow.

Current workflows invariably require troublesome steps such as preheating and handling small volumes of hot liquids in a time- and temperature-sensitive manner. These steps are the laboratory pain points that make hybridization-capture cumbersome to execute and challenging to automate. Optimum performance generally necessitates overnight hybridization and high library inputs. Shorter hybridization times on existing platforms negatively impact targeting, coverage, and GC-balance. The high library input mandates additional PCR cycles that can lead to increased duplication rate and can complicate variant calling.

Exome: IDT xGen™ Exome Hyb Panel v2
IDP: IDT xGen™ Inherited Diseases Hyb Panel
AML: IDT xGen™ AML Cancer Hyb Panel

All representative performance illustrated were performed with 150bp-sheared NA12878 reference libraries prepared from IDT xGen™ cfDNA & FFPE DNA Library Prep Kit

High performance with short hybridization incubations

Current workflows suffer lower on-target, higher GC-skew, and lower coverage with shortened hybridization incubations. The new workflow performance is less sensitive to shorter hybridization and often surpasses overnight hybridization performance of current workflow.

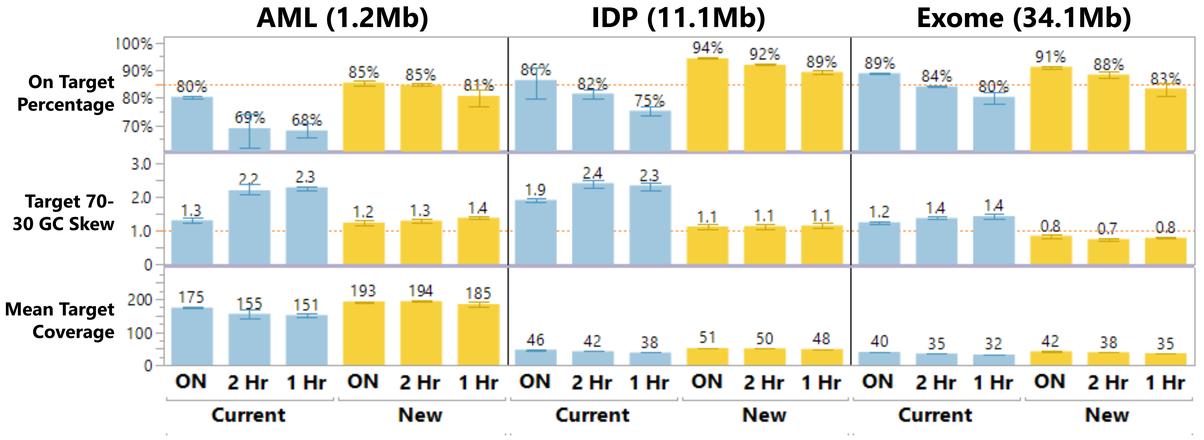


Figure 2: Hybridization incubation time. Incubation time and workflow are shown on the x-axis. 4 technical replicates per condition. 500ng library input per sample. Reads/sample: AML 4M; IDP 10M; Exome 30M

High performance with low hybridization-capture library inputs

The new workflow at low library inputs and low hybridization durations show equivalent performance to conventional 500ng library input and overnight hybridizations. No additional PCR cycles were needed for low inputs and hybridization durations.

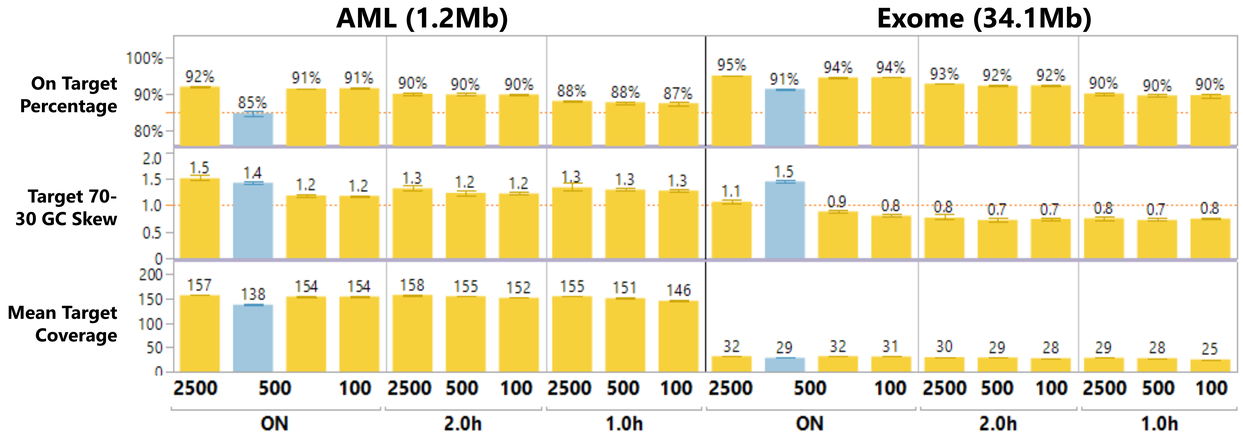


Figure 3: Hybridization library input. Representative performance shown for small and large panels: Total library input, hybridization time, and probe panels are shown on the x-axis. 4 technical replicates per condition. Reads/sample: AML 4M; Exome 23.4M

Higher coverage with same number of reads

The new workflow exhibit same coverage for short and overnight hybridization for small to large probe panel sizes. The new workflow provides more coverage for the same number of reads for all conditions.

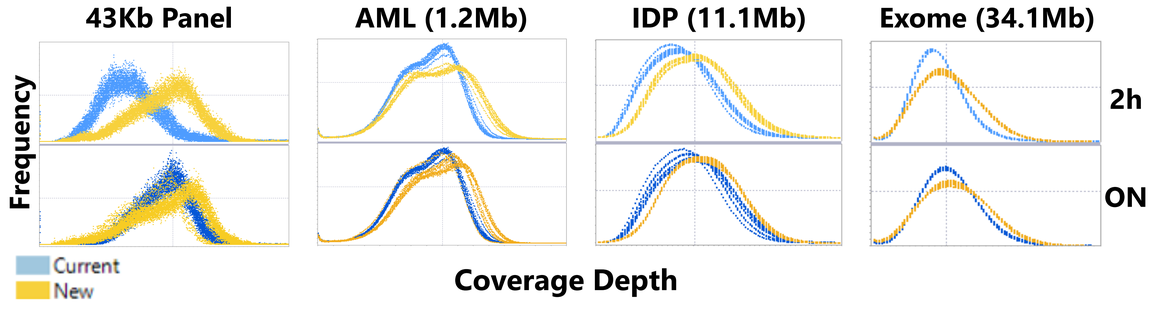


Figure 4: Target coverage. The 2-hour (2h) and Overnight (ON) hybridizations are plotted on the same x- and y-scale for each probe panel without normalization. Coverage increases from left to right on x-axis for each of the panels shown. Each condition contains samples from multiple experiments: 12 samples per condition. Reads/sample: 43Kb Panel 1M; AML 5M; IDP 10M; Exome 30M