

Introduction

Targeted sequencing by hybridization-capture is a versatile and powerful way to interrogate selected regions of interest in large and complex genomes. However, the performance of a given hybridization-capture workflow is often optimized only towards a specific approach to probe panel design. Here we demonstrate a hybridization-capture enrichment system that delivers high performance on three commercially available Exome panels with short and overnight hybridizations. This is achieved using our new xGen[™] Hyb and Wash v3 (v3), which features a simple workflow without any pre-heating, hot-liquid handling, or time-and-temperature sensitive steps. Unlike currently existing workflows, v3 steps are decoupled; heating is not sensitive to ramp rate and samples can reach room temperature after heated steps with no impact on performance.

Method

Human genomic reference libraries were constructed with xGen cfDNA & FFPE DNA Library Preparation Kit using 25 ng Coriell NA12878 gDNA sheared to 150 bp. The adapter-ligated fragments were pooled and re-divided for indexing to minimize library variations. These equivalent libraries are targeted using xGen Exome Hyb Panel v2 (Exome v2), xGen Exome Hyb Panel v1 (Exome v1), or a double-stranded-probe Exome panel (dsExome) with 1 hour or overnight (ON) hybridizations. All representative performance consist of ≥ 3 technical replicates/condition. All 100–1000 ng hybridizations are singleplex. All 2.5 μ g hybridizations are 5-plex at 500 ng/index. Benchmarking using the currently available xGen Hyb and Wash v2 (v2) is presented for performance comparison. Post capture libraries were sequenced on the Illumina[®] NextSeq2000 with low pass sequencing.

Easy workflow with fewer components

xGen Hyb and Wash	v2	v3
Minimum recommended library total input	500 ng	100 ng
Maximum recommended library total input	6000 ng	6000 ng
Minimum recommended hybridization time	4 hours	1 hour
Wash buffers to mix/dilute	6	1
Capture and wash hands-on steps	17	9
*Hot liquid handling steps	7	0
*Hot buffers to pre-heat	3	0
Incubations	6	3
Lab & user-dependent: estimation for 32 samples Manual Processing (min)	117	82

***Workflow pain point**

Table 1. Comparison of the current and new xGen Hyb and Wash. The v3 workflow features a shortened protocol with decoupled execution steps that entail fewer hands-on steps, fewer reagents, no pre-heating, no hot-liquid handling, and more flexible inputs and hybridizations.

xGen Hyb and Wash v3 performs equivalently with all 3 Exome panels for all inputs and hybridizations

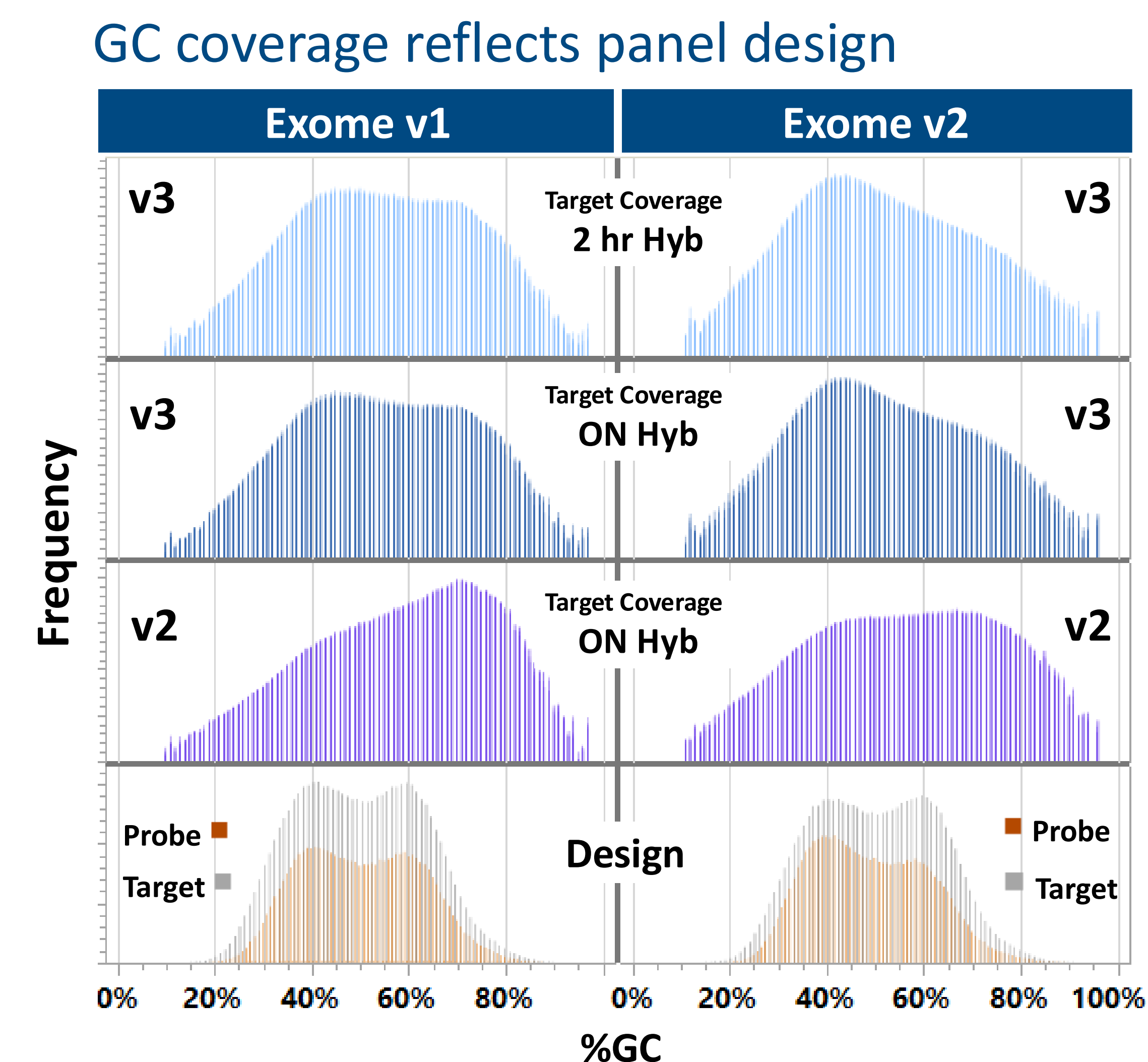
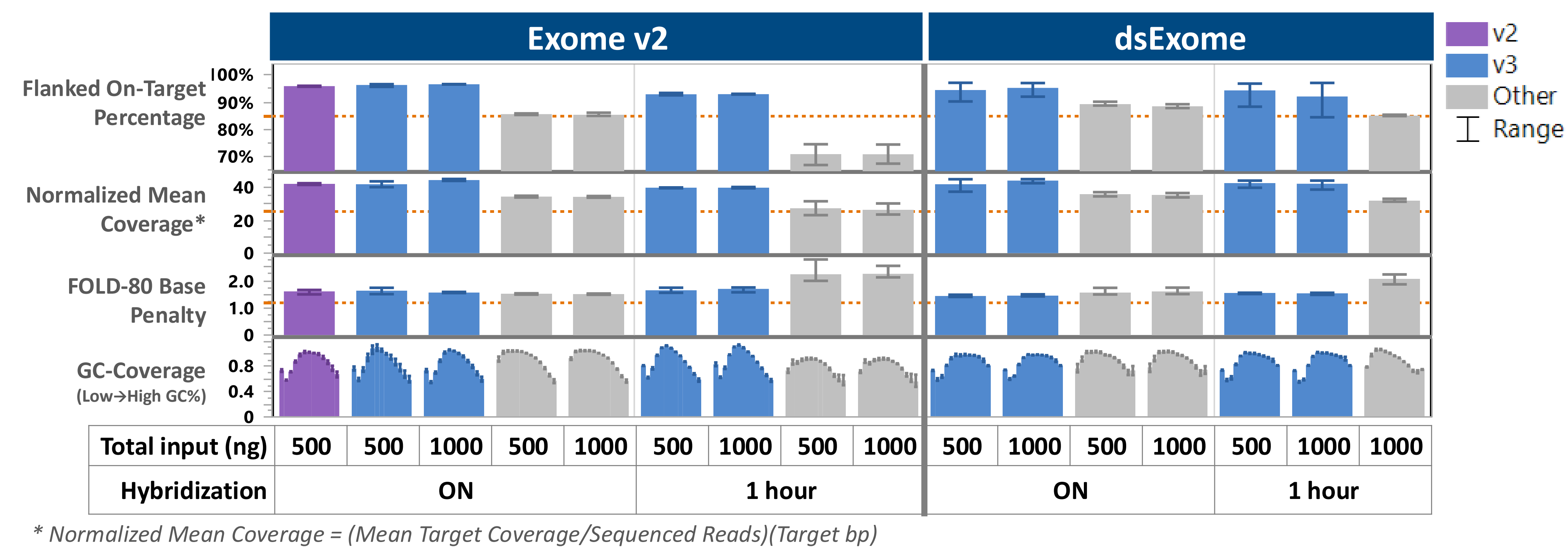


Figure 1. v3 GC coverage reflects probe panel design. Bottom row illustrates GC-content of the target at 100 bp bins and probe at 120 bp/probe. Top 3 rows illustrate the GC content of resulting coverage in 100 bp bins. 500 ng total input library per hyb-capture are shown for each hyb-capture condition. Each coverage plot contains 4 technical replicates per condition at 23.4 M reads per sample.

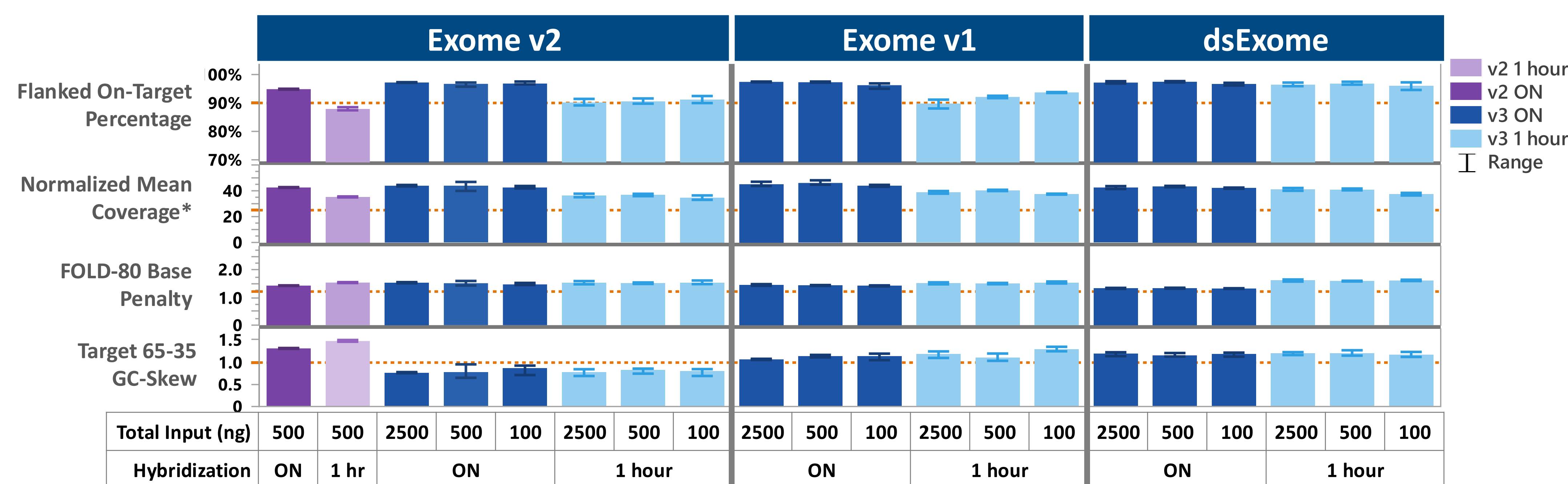
Simple workflow delivers superior performance on different design approach and probe panel format

Figure 3. xGen v3 compatibility with different panel designs. Each bar illustrates averaged value of 4 technical replicates per condition with 12.5 M reads per sample. Bar bracket shows range of actual sample values per condition. Flanked On-Target is defined as ± 150 bp from target. Superior targeting and coverage performance of v3 is conserved for multiple types of designs and panels, for all inputs and hybridization durations. The ‘Other’ workflow delivered relatively consistent performance on dsExome for overnight and 1-hour hybridizations. Its performance is significantly compromised with panel and design from a different source. This illustrates v3 flexibility in delivering high performance across design approaches and probe panel format, demonstrating an important value for applications that require custom designs and spike-in probes.



^{*} Normalized Mean Coverage = (Mean Target Coverage/Sequenced Reads)/(Target bp)

Simple workflow performs consistently across inputs, hybridization durations, and panel design



^{*} Normalized Mean Coverage = (Mean Target Coverage/Sequenced Reads)/(Target bp)

Target coverage correlations show conserved behavior for 1-hour and overnight hybridizations

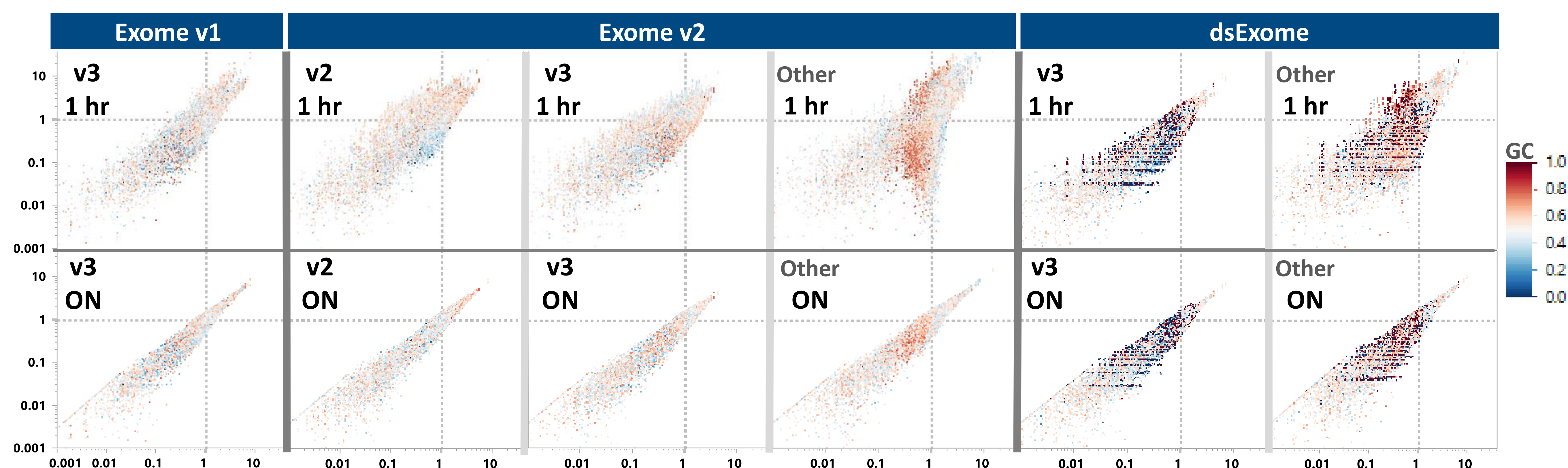


Figure 2. Target coverage correlation with averaged overnight hybridization for each panel and workflow with 1-hour (1 hr) or overnight (ON) hybridizations. Designs consist of 19.8 K to 32.1 K targets per panel, up to >30 Kbp per target. Coverage values are normalized by each sample’s Mean Coverage. Each target is represented by a single dot colored by its GC content. Three (Exome v1) or four (Exome v2 and dsExome) technical replicates are shown for each condition on the y-axis. Different batches of libraries were used for each of the panels displayed here. Each pair of 1 hour and overnight hybridizations consist of the same library batch. The x-axis consists of the averaged value of the ON hyb for each condition. Both x-and y-axis are log 10 scale. xGen Hyb Wash v2 was performed for Exome v2 as a baseline. ‘Other’ workflow is the workflow that accompanies the dsExome panel. 500 ng input library per hybridization for Exome v1 and v2. 1000 ng, as recommended by the supplier, per input library for dsExome. Post-capture amplification for the dsExome panel was performed with dsExome supplier PCR mix. Bottom row illustrates the noise spread against the mean of the same sample set. Top row illustrates change in target coverage with short hybridization. IDT v3 workflow showed similar coverage trend for long and short hybridizations for all 3 Exome panels. The ‘Other’ workflow exhibits sensitivity to panel design. Non-biased sampling reveals properties of the library as directed by the panel design.

Summary

New workflow delivers consistent coverage and high performance with:

- Different panel design approaches, including single- and double-stranded probes.
- Low to high total library inputs.
- Short and long hybridizations.
- Simplified and easy enrichment.

The demonstrated flexibility delivers fidelity to panel design in routine assays as well as applications that require custom panels, spike-ins, multiplexing, and limited library quantities.

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