Target enrichment via in-solution hybrid capture using xGen™ Lockdown™ Probes yields >5000-fold enrichment, excellent uniformity, and virtually no GC bias. Lockdown Probes are suitable for supplementing existing array-derived capture panels or creating entire custom standalone panels.

**Overview**

IDT xGen Lockdown Probes were assessed as a stand-alone target enrichment panel and for supplementing an array-derived RNA capture panel. Target enrichment was performed by both methods using ligation-based sequencing libraries that were prepared and amplified using HiFi polymerase (Kapa Biosystems).

For standalone enrichment, a 133 kb region of the human genome, representing 57 clinically-relevant, potentially actionable cancer-related genes was analyzed. Using 2 µg target DNA input and 1000 Lockdown Probes, in-solution hybrid selection was performed in a 24 hr hybridization. Captured fragments were isolated using streptavidin. Enriched libraries were sequenced on a HiSeq 2000 system (Illumina) using 49 x 49 paired-end reads.

Alternatively, a 1.1 Mb array-derived, RNA-based capture panel was supplemented with the same 1000 Lockdown Probes to improve coverage of GC rich targets. Again, enriched samples were sequenced as 49 x 49 paired-end reads using an Illumina HiSeq™ 2000 sequencing platform.

When xGen Lockdown Probes were as standalone probes, approximately 5000-fold enrichment was achieved with high coverage (Figure 1) and minimal GC bias (Figure 2) over the entire 133 kb region. Additionally, insertions and deletions (indels) were detected at the expected frequencies in DNA from tumor cell lines enriched using Lockdown Probes (Figure 3).

Supplementing array-derived RNA baits with 1000 Lockdown Probes enhanced the coverage of many GC-rich regions, such as first exons (Figure 4).
Improved Target Selection Using xGen™ Lockdown™ Probes

Figure 3. Reliably Detect Insertions and Deletions. Expected allele frequency is compared to observed frequency after analysis of NGS sequencing data from a target enriched with xGen™ Lockdown™ Probes. Hybrid capture with Lockdown Probes enabled detection of insertions (between 1 and 35 bases) and deletions (between 1 and 36 bases) at the expected frequencies.

Figure 4. Improved Capture Performance by Supplementing Existing Panels. Capture of a 1.1 Mb region using array-derived RNA baits resulted in regions of poor coverage (top). Supplementing the array-derived capture panel with xGen™ Lockdown™ Probes led to improved coverage of these regions and more uniform coverage overall (bottom).

Conclusions
- Excellent capture of very small (<25 kb) target regions
- High target enrichment (>5000 fold)
- Minimal GC bias for GC content in the range 20–80%
- Ultra-deep coverage of the entire targeted region
- Efficient and reliable capture of indel-containing alleles
- Improve performance of array-derived bait pools
- Low cost per sample

About xGen™ Lockdown™ Probes
xGen Lockdown Probes are individually synthesized 5’ biotin modified DNA oligonucleotides based on the Ultramer™ synthesis platform. Probes can range from 60-120nt to allow for customized design parameters, and are available in 3 synthesis scales to support high volume applications.

- Probes are individually assessed by mass spectrometry for QC
- All probes contain a 5’ biotin modification
- Probes are pooled in equimolar quantities
- Standard and XL scale probes can be delivered in plates
- Probes are shipped in 7–10 days

<table>
<thead>
<tr>
<th>Scale</th>
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* Based on 500 ng of pooled probes per capture.

For more information about xGen™ Lockdown™ Probes, visit www.idtdna.com/xgen or email xgen@idtdna.com.