

Improved Targeted Sequencing with xGen™ Lockdown™ Probes

Data contributed by Foundation Medicine, Inc., Cambridge, MA

Introduction

Target enrichment via in-solution hybrid capture using xGen Lockdown Probes yields high enrichment levels and uniformity with virtually no GC bias. Lockdown Probes can be used to both supplement an existing capture panel derived from arrays, or to create entire custom standalone panels.

Overview

Standalone enrichments as well as a supplementation of an RNA capture have been performed using xGen™ Lockdown™ Probes using RNA-based reagents. Ligation-based sequencing libraries were prepared and amplified using HiFi polymerase from Kapa Biosystems. Two standalone captures were performed using 2 µg of input and 1,000 Lockdown probes targeting 133kb. Hybridization time for this capture was 24 hours. A subsequent experiment was performed in which a 1.1Mb RNA-capture panel was supplemented with the 1,000 Lockdown probes to improve coverage over GC rich targets. All samples were sequenced using an Illumina HiSeq™ 2000 sequencing platform.

Summary of Performance

- Excellent performance for very small target areas
- High levels of enrichment (5,000+ fold)
- Minimal GC bias between 20 and 80% GC content
- Ultra-deep coverage of the entire targeted region
- Efficient and reliable capture of IN/DEL containing alleles
- Improved performance of array-derived bait pools
- Low per sample cost

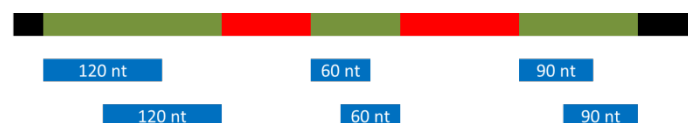
About xGen™ Lockdown™ Probes *beta*

xGen Lockdown Probes are individually synthesized 5' biotin modified DNA oligos 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.

Scale	Yield	Min Order	Price/Probe
Mini	2 pmole	200 probes	\$15
Standard	20 pmole	96 probes	\$25
XL	200 pmole	96 probes	\$35

- 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

Flexible Probe Lengths to Accommodate Unique Designs



Example: 2X tiling with variable probe length with no tiling in repeat or off target regions.

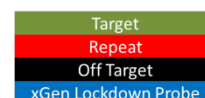


Figure 1. The ability to synthesize probes between 60-120nt allows for flexible design strategies to accommodate higher target specificity or deeper coverage in different regions.

Visit www.idtdna.com/pages/xgen or email xgen@idtdna.com for questions regarding xGen™ Lockdown™ Probes.

Performance

Reliably Detect Rare Alleles with Smaller Custom Panels

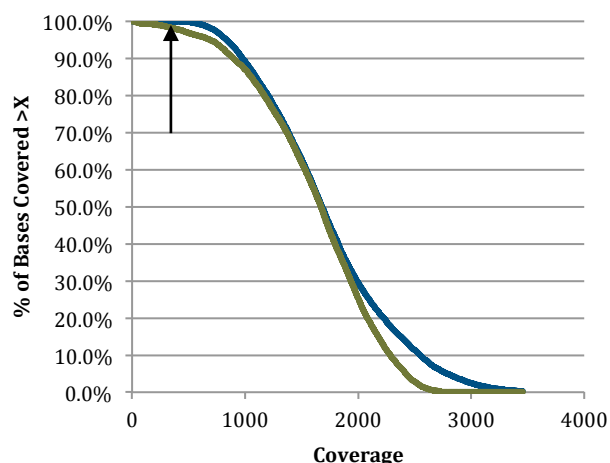


Figure 2. A 133 kb capture using xGen Lockdown Probes (blue) is compared against an equivalent portion of a 1.1 Mb capture using array-derived RNA baits (green). The arrow shows that 99.9% of all target bases were covered at >500X for Lockdown probe captured baits versus 96.9% for RNA baits.

Detect Insertions and Deletions with High Reliability

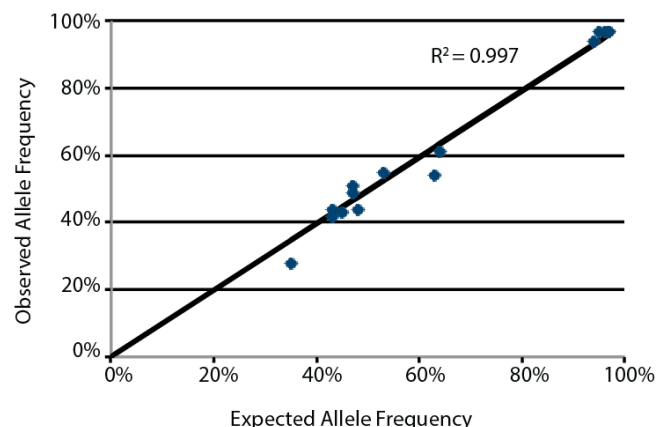


Figure 4. The expected allele frequency is compared against observed frequency after analysis of a NGS sequencing run enriched with xGen™ Lockdown™ Probes. Hybrid capture with Lockdown Probes enabled detection of insertions (between 1 and 35 bases) and deletions (between 1 and 36 bases) at the expected frequencies.

Achieve Minimal GC Bias

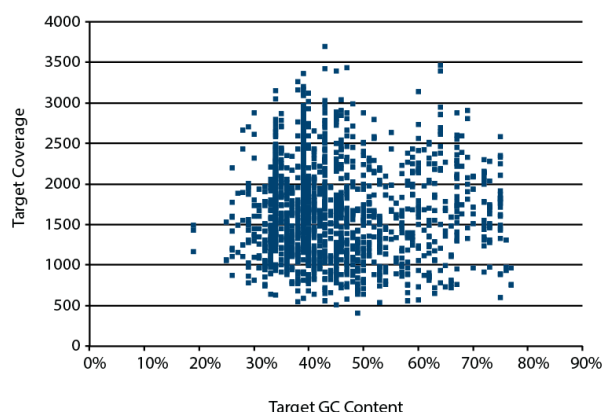


Figure 3. The GC content of target sequences is compared to the total coverage against those sequences. Little systematic biases were observed in relation to target GC content when using Lockdown probes as a standalone capture.

Improve Performance by Supplementing Existing Captures

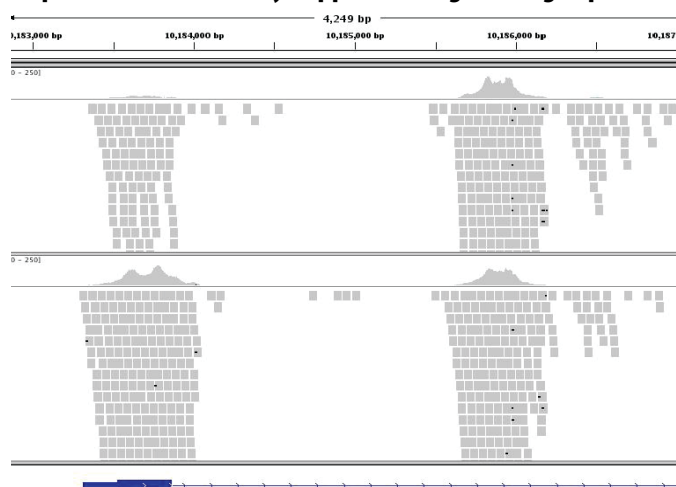


Figure 5. A standalone 1.1 Mb RNA capture panel (top) contains regions of poor coverage. Supplementing the capture panel with xGen Lockdown Probes (bottom) resulted in improved coverage against those regions and more uniform coverage overall.