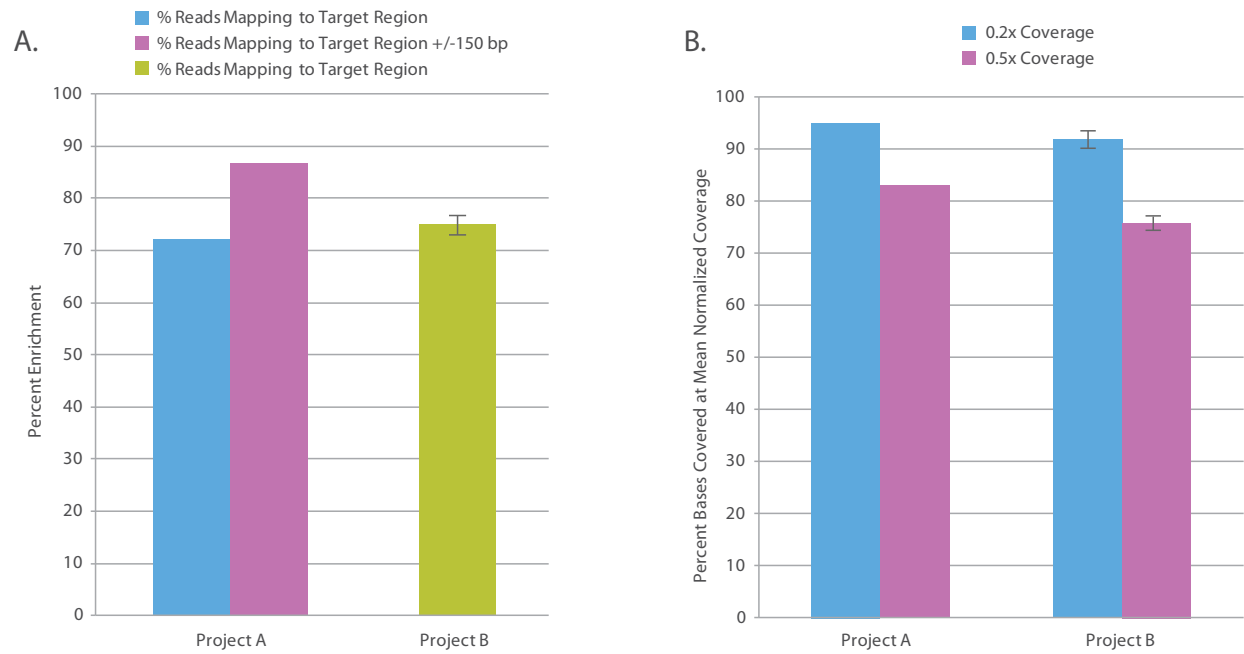






Figure 4: High Target Specificity and Coverage Uniformity



A. Percent enrichment, defined as reads mapping to target regions out of total reads per run, is shown for two example custom enrichment projects. For single-plex Project A, > 70% enrichment is achieved for reads mapping exactly to target regions (blue). An increase to > 85% is observed when the regions are expanded to +/- 150 bp surrounding the targeted coordinates (purple). For 12-sample multiplexed Project B, the mean percent enrichment averages ~75%, (green) are shown. B. Mean normalized coverage plots for the same two example projects show that > 90% of bases are covered at 0.2x of the mean coverage (blue bars), and > 75% of bases are covered at 0.5x of the mean coverage in both projects (purple bars).

These key parameters and a method for precalculating the amount of sequencing and mean coverage required to fully optimize any targeted sequencing study is described in greater detail in the Optimizing Coverage for Targeted Resequencing Technical Note.<sup>2</sup>

## Data Examples

Two different TruSeq Custom Enrichment experiments were performed following the workflow described in Figure 1. Each project included different target regions, plexities, library sizes, target region sizes, probe interval spacing, and coverage depths (Table 1). Representative enrichment and coverage data are shown in Figure 4. Project A employed single-plex targeting of ~2 Mb total sequence with 20,000 attempted probes. Project B used a 12-plex strategy to target ~1.0 Mb of total sequence with 6,200 attempted probes. Both projects used gel-free TruSeq DNA Sample Preparation Kits prior to enrichment, and were sequenced using a Genome Analyzer<sub>IIx</sub>. In both single- and multiplexed projects, high percent enrichment in targeted and padded regions was achieved, shown in Figure 4. For both projects, mean normalized coverage plots show that > 90% of bases are covered at 0.2x of the mean coverage, and > 75% of the bases are covered at 0.5x of the mean coverage.

Table 1: TruSeq Custom Enrichment Project Details

Detail	Project A	Project B
Unique Bases Targeted	~2.2 Mb	~1.0 Mb
Multiplex Level	1	12
Library Size	350 bp	400 bp
Full Region/Exon	Full Region	Exons
Probe Interval Spacing	Dense	Dense
Reference Sequence	UCSC hg19	UCSC hg19
Total Probes	~20K	~6.2K
Percent Enrichment*	72/87	~75
Percent Bases Covered**	95/83	91/75
Avg. Sequencing Depth†	47x	100x

\*Percent enrichment shown as mapped only to target regions/mapped to target regions +/-150 bp (Project A), and to exons (Project B).

\*\*Percent bases covered, shown as mean normalized coverage plots.

†Sequenced on the Genome Analyzer<sub>IIx</sub>.

