

Sequencing depth requirements for xGen™ HS EGFR Pathway Amplicon Panel

Molecular IDs (MIDs) or unique molecular identifiers (UMIs) help to prevent sequencing errors by confirming true low allele frequency variants. However, to utilize MIDs or UMIs appropriately, a minimum sequencing depth is required. A singleton read is a read present only once in a sequence, and most likely contains at least one error. A high frequency of singleton reads can result in wasted sequencing efforts due to a lack of error correction to confirm true low-frequency variants in a sequence. **Figure 1** and **Table 1** below provide details on how sequencing depth improves the reliability of calling known low frequency variants (0.5%) with the xGen HS EGFR Pathway Amplicon Panel.

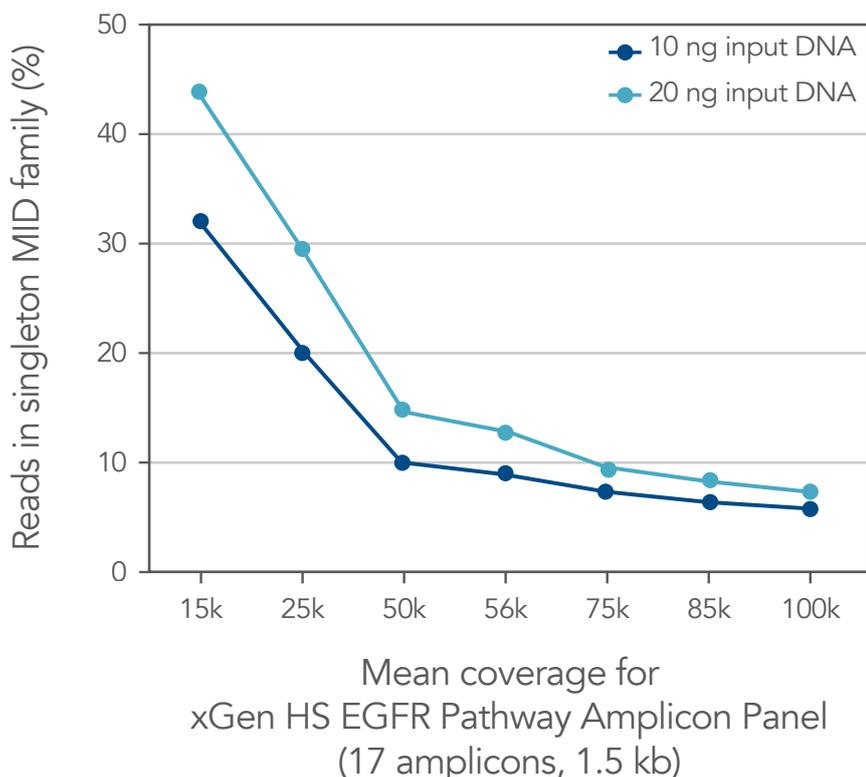


Figure 1. A sequencing depth below 55k indicates a higher percentage of the reads were singletons. Libraries (n=2) were generated from 10 ng and 20 ng SeraCare® DNA and sequenced on a MiniSeq® (Illumina®) instrument at a 2 x 150 bp read length. Sequence data were down sampled to represent different sequencing depths. Data shows the percentage of singleton reads at each sequencing depth, also referred to as mean amplicon coverage.

Sequencing data from libraries (n=2, 10 ng) were down sampled to various depths, to compare the mean amplicon coverage to the mean MID-based coverage (**Table 1**). As sequencing depth increases, the MID-based amplicon coverage also increases, and the percent of known variants at 0.5% (n=7) allele frequency can be confirmed.

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Table 1. Sequencing data from libraries (n=2, 10 ng) were down sampled to various depths to show the amplicon coverages pre- and post-MID error correction.

Number of reads (M)	Mean "raw" amplicon coverage	Mean "MID-based" amplicon coverage	% Known variants called (0.5% allele frequency)
0.26	15,000	1,682	63
0.44	25,000	3,528	63
0.88	50,000	7,033	88
1.00	56,789	7,728	100
1.32	75,000	9,149	100
1.50	85,139	9,764	100
1.76	100,000	10,468	100

Technical support: applicationsupport@idtdna.com

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