Sequencing coverage describes the average number of reads that align to, or "cover," known reference bases. The next-generation sequencing (NGS) coverage level often determines whether variant discovery can be made with a certain degree of confidence at particular base positions.

Sequencing coverage requirements vary by application, as noted below. At higher levels of coverage, each base is covered by a greater number of aligned sequence reads, so base calls can be made with a higher degree of confidence.

Most users determine the necessary NGS coverage level based on the application, as well as on other factors such as size of reference genome, gene expression level, published literature, and best practices defined by the scientific community.

Examples of sequencing coverage recommendations for some common applications include:

- **For detecting human genome mutations, SNPs, and rearrangements**
  publications often recommend from 10× to 30× depth of coverage, depending on the application and statistical model. We usually recommend 15x for homozygous SNV, 30x for heterozygous SNV and 60x for INDELs.
- **For RNA sequencing**
  researchers usually think in terms of numbers of millions of reads to be sampled. Detecting rarely expressed genes often requires an increase in the depth of coverage. We usually recommend 10-25M single-end reads for mid-high expressed genes and 50-100M single-end reads for low gene expression or SNP calling. Use paired-end reads for de novo transcriptomic analysis or detecting splicing events. Also, use at least 3 biological replicates for each condition.
- **For ChIP-Seq (chromatin immunoprecipitation sequencing)**
  publications often recommend coverage of around 100x. Usually 10-15M single-end reads for transcription factor analysis and 20-40M for histones.
- **For Small RNA or miR-seq**
  usually 1-5M single-end reads is sufficient. Small read length is standard <50nt.
- **For CRISPR library validation**
  a good rule of thumb is to have >100 reads per sgRNA. Contact us before starting your library preparation as some factors as be taken into considerations.

To estimate and achieve your desired NGS coverage, please contact us in advance in order to have a better idea of your experimental design and a cost estimate. You can always use these tools to guide you:

Illumina Coverage Calculator:
https://support.illumina.com/downloads/sequencing_coverage_calculator.html

RNAseq length and reads:
https://support.illumina.com/bulletins/2017/04/considerations-for-rna-seq-read-length-and-coverage-.html

Coverage estimator by application:
https://genohub.com/recommended-sequencing-coverage-by-application/