

There are 2 main strategies for indexing/barcoding (which are the same thing for our purposes here).

INLINE

The Inline barcodes are adjacent to the sample DNA and read from the same sequencing primer as part of the same sequence read as the insert. They are obligatory (must be read) and they need to be handled post-image-processing by bioinformatic analysis. They also take up some of the sequencing read length. On the plus side, they are easy to add, cheaper to analyze/run, and usually faster to process.

MULTIPLEX

The Multiplex indices are in the stem or arm of the library. Reading them is optional, but requires additional priming and sequencing so it is slightly more expensive and takes longer. On the plus side, the image pipeline generally bins/sorts them for you and you don't give up any space in your sequencing read.

HOW TO CHOOSE

Multiplex indices are recommended for short reads where every base is needed and for working with unannotated or newly acquired genomes. Inline barcodes are recommended for long reads of well-annotated genomes such as Chip-Seq, SNP, or transcriptome analysis. You can combine both approaches if you need more degrees of variation. Inline barcodes should be non-redundant and base-balanced as much as possible. The Multiplex indices are less affected by base-bias, but care should be taken to avoid base-overlap to prevent "Index Switching", a phenomenon that occurs when a mis-called base causes a read to be binned to the wrong index. If you have more than 12 libraries in your mix, using an 8-base or 10-base index will allow for mis-matches and still let the index be called correctly. Dual indices are strongly recommended.

Below are the 12 barcodes used in the Illumina TruSeq system, they are base-balanced and work well as In-Line barcodes as well as Multiplex.

ATCACG	CGATGT	TTAGGC	TGACCA	ACATGT	GCCAAT
CAGATC	ACTTGA	GATCAG	TAGCTT	GGCTAG	CTTGTA

Many sample prep kits are available from Illumina or third-party vendors, but stand-alone oligos can also be purchased from IDT or similar vendors.

Depending on your library prep chemistry, remember to add a T at the end of the adapter when designing oligos if your ligation step uses an A-tailed reaction.

