What sequences do I use for adapter trimming?

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When performing sequencing on an Illumina instrument, sequences corresponding to the library adapter can be present in the FASTQ files at the 3' end of the reads if the read length is greater than the insert size. To remove these sequences and prevent issues with downstream alignment, adapter trimming is an option in Illumina FASTQ generation pipelines. Sample sheets generated with Illumina Experiment Manager contain the necessary sequences in the [Settings] section for Illumina kits. Illumina kits in both BaseSpace Sequence Hub Prep tab and Local Run Manager have adapter information built in. However, some third-party tools require the adapter sequence for trimming be specified separately. The recommended sequences to use for each Illumina kit are as follows.

TruSeq LT and TruSeq HT-based kits:

- Read 1: AGATCGGAAGAGCACACGTCTGAACTCCAGTCA
- Read 2: AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT

ScriptSeq and TruSeq DNA Methylation:

- Read 1: AGATCGGAAGAGCACACGTCTGAAC
- Read 2: AGATCGGAAGAGCGTCGTGTAGGGA

TruSeq Small RNA:

• TGGAATTCTCGGGTGCCAAGG

TruSeq Ribo Profile:

AGATCGGAAGAGCACACGTCT

AmpliSeq, Nextera, Nextera DNA Flex, Nextera DNA, Nextera XT, Nextera Enrichment, Nextera Rapid Capture Enrichment, TruSight Enrichment, TruSight HLA:

• CTGTCTCTTATACACATCT

Nextera Mate Pair:

• CTGTCTCTTATACACATCT+AGATGTGTATAAGAGACAG

Notes:

- Adapter trimming is not required for TruSeq Targeted RNA Expression, TruSeq Custom Amplicon, and TruSeq Cancer Panel when using Illumina analysis pipelines. Adapter sequences for TruSeq Synthetic Long Reads are proprietary and removed during assembly.
- For TruSeq Small RNA and Ribo Profile, only one sequence is provided as single-read runs are recommended. For Nextera/TruSight, the same sequence is used for both reads.
- For more information on adapter trimming in Nextera Mate Pair, see the Data Processing of Nextera Mate Pair Reads on Illumina Sequencing Platforms technical note.
- The full adapter sequences for various Illumina library preparation kits can be found in the Illumina Adapter Sequences Document.

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