



## NEXTflex™ Small RNA Trimming Instructions

Sequencing reads generated with the NEXTflex Small RNA Seq Kit v3 require trimming of adapter sequences and random bases prior to alignment when using and end-to-end alignment mode. These trimming steps can be accomplished using cutadapt (<https://cutadapt.readthedocs.io/en/stable/>), which is free to download.

The following commands require the latest version of cutadapt, which can be installed using the directions found at <http://cutadapt.readthedocs.io/en/stable/installation.html>.

1. Trim 3' adapters:

```
cutadapt -a TGGAATTCTCGGGTGCCAAGG -o YOUR_FILE.trim1.fq --minimum-length 23 YOUR_FILE.fastq.gz
```

This command excludes any inserts less than 15 bases (where the minimum length of 23 refers to 8 randomized bases and a minimum 15-base insert). It takes as input “YOUR\_FILE.fastq.gz” and writes out to “YOUR\_FILE.trim1.fq”.

2. Use this command to trim 4 bases from either side of each read:

```
cutadapt -u 4 -u -4 -o YOUR_FILE.trim2.fq YOUR_FILE.trim1.fq
```

From here, you can align “YOUR\_FILE.trim2.fq” to an appropriate reference using an aligner such as bowtie2.