NEXTFLEX®

Small RNA-Seq Kit v4

Product number: NOVA-5132-31; -32; -41-44

NEXTFLEX SMALL RNA TRIMMING INSTRUCTIONS

Sequencing reads generated with the NEXTFLEX Small RNA Seq Kit v4 require trimming of adapter sequences prior to alignment when using and end-to-end alignment mode. These trimming steps can be accomplished using cutadapt (https://cut- adapt.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′ adapter:
   cutadapt \
   --cores 0 \
   --quality-cutoff 20 \
   --adapter TGGAATTCTCGGGTGCCAAGG \
   --output YOUR_FILE.cut.fastq \
   --minimum-length 16 \
   YOUR_FILE.fastq.gz
   This command excludes any inserts less than 16 bases and removes bases from the 3′ end of the insert with a quality score less than 20. Adjust values for minimum length and quality score as desired. It takes as input “YOUR_FILE.fastq.gz” and writes out to “YOUR_FILE.cut.fastq”.

2. Trim 3′ and 5′ adapter from paired-end reads:
   cutadapt \
   --cores 0 \
   --pair-adapters \
   --quality-cutoff 20 \
   --adapter TGGAATTCTCGGGTGCCAAGG \
   -A AGATCGGAAGAGCGTCGTGTAGGGAAAGA \
   --output YOUR_FILE.cut.R1.fastq \
   --paired-output YOUR_FILE.cut.R2.fastq \
   --minimum-length 16 \
   YOUR_FILE.R1.fastq.gz \
   YOUR_FILE.R2.fastq.gz
   This command is similar to the single end version but requires paired reads to contain both adapters. If that is not the case, remove the “--pair-adapters” flag. If you would like to set a different quality score cutoff for read 2, use “-Q [value]” with “value” equal to the desired read 2 quality cutoff.

From here, you can align your trimmed fastqs to an appropriate reference using an aligner such as bowtie2.

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