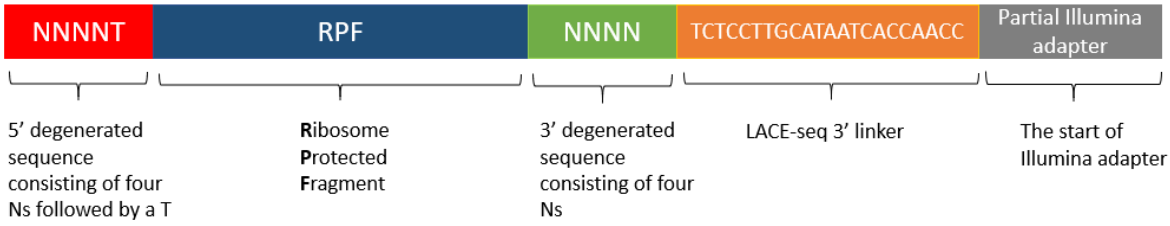
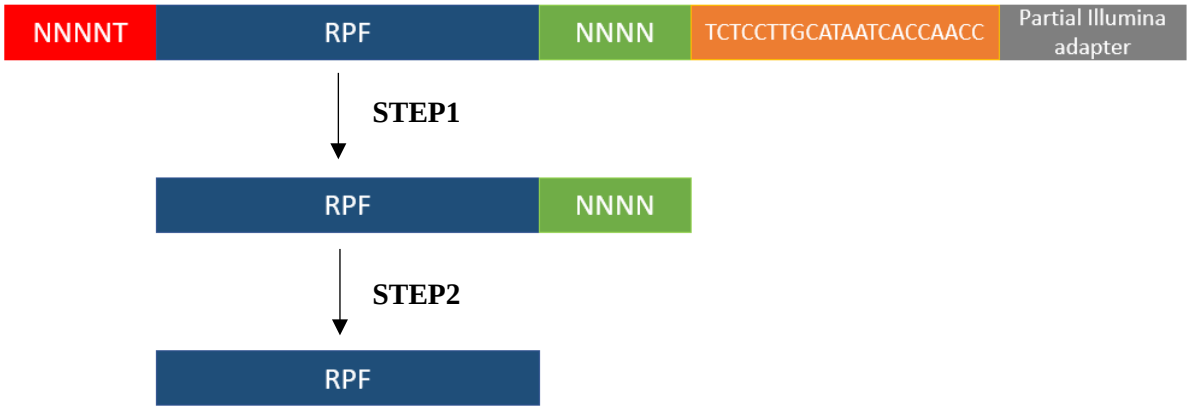
Supplemental product information and tips for success

Example of read generated by LACE-seq for Illumina:



Proper trimming of the reads is important for efficient mapping. Here we provide some guidance on the use of cutadapt (Martin, 2011) to trim reads generated by LACE-seq for Illumina.

Trimming is done in 2 steps:



If cells were treated with CHX and processed with Ribolace module 1, reads between 28 nt and 35 nt are expected to show the best 3nt periodicity.

STEP1

First the LACE-seq linker is trimmed from the 3' end of each read and the first five nucleotides corresponding to the 5' degenerated sequence are removed from the 5' end:

```
cutadapt --cut 5 -a TCTCCTTGCATAATCACCAACC --discard-untrimmed -o
trim1.fastq input.fastq
```

Parameter	Definition
`--cut 5`	Removal of the first 5 bases from the beginning of each read
`-a TCTCCTTGCATAATCACCAACC`	Removal of the LACE-seq 3' linker and any sequence that may follow
`--discard-untrimmed`	Reads in which *no* adapter is found are discarded
`-o trim1.fastq`	The output file name
`input.fastq`	The input file name

STEP2

The 3'degenerated sequence is then removed from the 3'end of each read, and only reads longer than **X** nt are retained, while shorter are discarded:

```
cutadapt --minimum-length X --cut -4 -o trim2.fastq trim1.fastq
```

Parameter	Definition
`--minimum-length X`	Reads above X nt are retained (usually X=20 for ribosome profiling analysis)
`--cut -4`	Removal of the last 4 bases at the end of each read
`-o trim2.fastq`	The output file name
`trim1.fastq`	The input file name must be the same as the output file name in step1