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dsRNAs treatment with RNase T1 and DNase I

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Protocol status: Working
We use this protocol and it's working

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Abstract

For dsRNA sequencing by nanopore sequencing, this protocol was used. Before treating samples with RNase T1, you should measure the total concentration of RNAs in the samples by using a nanodrop or Qubit device, as RNase T1 has the ability to partially digest double-stranded RNAs in the absence of single-stranded RNA.

Materials

00:00:00

1. DNase I (RNase-free)
2. DNase I Reaction Buffer (10X)
3. RNase T1

Digestion

30m

1 Add 10X DNase Buffer with MgCl2 (final concentration should be 1X).

2 Add 50 units RNase T1 per 1µg of total RNA and 1 unit DNase I per 2µg of total RNA

3 Incubate at 37 degrees C for 20 min.

Inactivation of enzymes

4 cleanup with phenol/chloroform extraction.