Rnase A treatment V.1

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This protocol is used to remove RNA from genomic DNA. RNase A is an endoribonuclease that specifically degrades single-stranded RNA at C and U residues. It cleaves the phosphodiester bond between the 5'-ribose of a nucleotide and the phosphate group attached to the 3'-ribose of an adjacent pyrimidine nucleotide. The resulting 2', 3'-cyclic phosphate is hydrolyzed to the corresponding 3'-nucleoside phosphate.

Rita Kuo 2021. Rnase A treatment . **protocols.io**
https://protocols.io/view/rnase-a-treatment-bz98p99w

protocol ,

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Disposables
- Tips
- Tubes
- Ice

Equipment
- Centrifuge
- Thermocycler
- Gel electrophoresis device
- Gel imager
- Nanodrop
- Pipettes
- Ice bucket

Reagent
- Rnase A (ThermoFisher EN0531)
- Nuclease free water
- Sodium acetate buffer solution (3M, pH 5.2) (ThermoFisher R1181)
- Ethanol, 200 proof
- SYBR Safe DNA gel stain (10,000x concentrate in DMSO)
- Gel Loading Dye
- DNA marker
- Ultra pure agarose

Rnase A treatment

1 Bring up the sample volume to 200 ul using TE buffer (pH 8.0)

2 Add 10 ul of RNase A

3 Incubate 37°C for 1 hour.

Sample QC

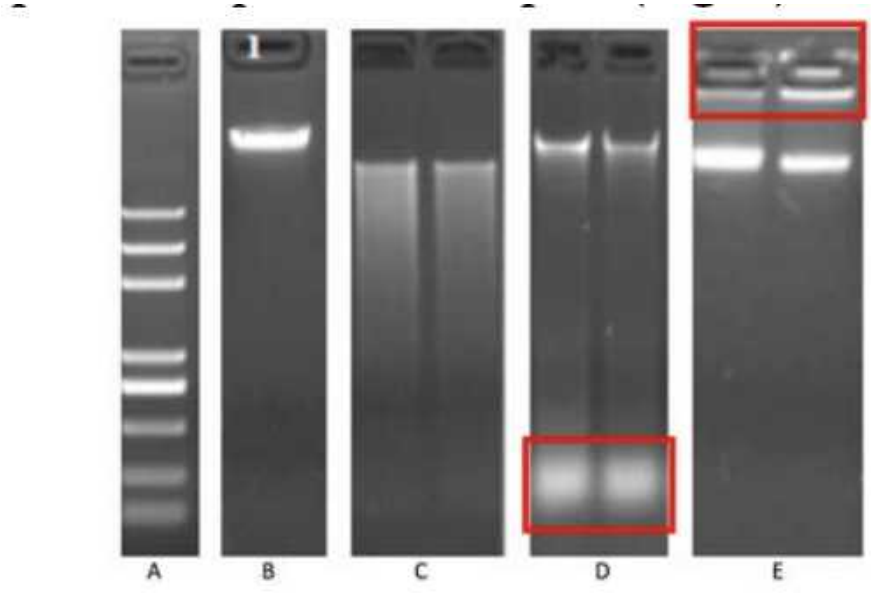
4 NOTE: Buffers and enzyme mix in the sample will affect Spectrophotometer reads. It's better to use gel to verify if sample still contains RNA.

Make 0.8 % agarose gel.

5 Load about 25ng/5 ul of sample on an agarose gel and use untreated sample as a control.

6 Run gel 10-15 minutes. If RNA is visible on the gel, add another 10 ul of RNase A and repeat the incubation step, otherwise proceed ethanol precipitation.

See the Lane D on following figure for an example of RNA contaminated sample.



Examples of DNA quality. (A) Trans2KTM Plus DNA Marker, (B) qualified sample, (C) degraded sample, (D) sample contaminated with RNA, (E) sample contaminated with protein. Red boxes denote areas of contamination. Figure adapted from PreQC-handbook-Novogene (https://jp.novogene.com/wp-content/uploads/sites/4/2020/04/PreQC-handbook-Novogene.pdf)

Ethanol Precipitation

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Add 1/10 volume of 3 M sodium acetate to RNase A treated DNA.

8 Add 2.5 X volumes of 100% ethanol

9 Gently mix and place it at -80°C for 30 minutes (or -20°C for overnight).

NOTE: Prepare 75 % ethanol and place it on ice before the next step.

10 Centrifuge at full speed (13000rpm), at 4°C for 20 minutes to pellet DNA.

11 Pour supernatant and wash pellet with freshly made 75 % **cold** ethanol.

12 Centrifuge at top speed at 4°C for 1 minutes.

13 Carefully remove ethanol with pipet tips

14 Suspend the DNA pellet with 30 ul (or appropriate volume) of nuclease free water .

15 Use Nanodrop for qualification and Qubit for quantification. The ratio of 260/280 should be 1.8 for DNA and a typical spectral pattern of DNA should look like this: