RNase A Cleanup of DNA Samples

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Summary

RNase A treatment is used for the removal of RNA from genomic DNA samples. RNase A 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.

Materials & Reagents

Materials	Vendor	Stock Number
Disposables		
1.5 ml microcentrifuge tubes		
Reagents		
TE Buffer, pH 8.0	Ambion	9849
RNase A, DNase and protease-free (10 mg/ml)	ThermoFisher	EN0531
Sodium acetate buffer solution (3M, pH 5.2)	VWR	567422
Ethanol, 200 proof	Pharmco-Aaper	111000200CSPP
50x TAE Buffer	Life Technologies/ Invitrogen	MRGF-4210
SYBR® Safe DNA gel stain (10,000x concentrate in DMSO)	Life Technologies/ Invitrogen	S33102
DNA Molecular Weight Marker II	Sigma	10236250001
Gel Loading Dye, Blue (6x)	New England BioLabs	B7021S
Equipment		
Centrifuge 4°C		
Heat block 37°C		
Gel electrophoresis device		
Gel Imager	Bio-Rad	

EH&S

PPE Requirements:

1.1 Safety glasses, lab coat, and nitrile gloves should be worn at all times while performing work in the lab during this protocol.

1.2 Ethanol is a highly flammable and irritating to the eyes. Vapors may cause drowsiness and dizziness. Keep containers closed and keep away from sources of ignition such as smoking.

Avoid contact with skin and eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Procedure

1. RNase A treatment
   1.1. Top off the DNA volume to 200 ul in TE buffer (pH 8.0)
   1.2. Add 10 ul of RNase A
   1.3. Incubate 37°C for 1 hour
   1.4. Check small aliquot (5 ul) on an agarose gel with no treatment control. Run gel 10-15 minutes. If there is no traceable of RNA, proceed to next step. If significant amounts of RNA are still present, add another 10 ul of RNase A and repeat the incubation
2. Ethanol Precipitation
   2.1. Add 1/10 volume of 3 M sodium acetate to RNase A treated DNA
   2.2. Add 2.5 volumes of 100% ethanol
   2.3. Mix and spin down sample
   2.4. Place at -80°C for 30 minutes (or -20°C for overnight)
   2.5. Spin sample at 4°C for 20 minutes to pellet DNA
   2.6. Carefully, pour off supernatant
   2.7. Wash pellet with 70°C ethanol (cold)
   2.8. Spin sample at 4°C for 3-5 minutes
   2.9. Take out all ethanol with pipet tips
   2.10. Air dry the pellet
   2.11. Resuspend pellet with 100 ul (or appropriate volume) of TE
   2.12. Quantify and qualify the DNA and store it at -80°C or -20°C

Change History