

Protocol

Fragmentation of DNA by Nebulization

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This protocol was adapted from “Commonly Used Techniques in Molecular Cloning,” Appendix 8, in *Molecular Cloning*, Volume 3, 3rd edition (eds. Sambrook and Russell). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, 2001.

INTRODUCTION

DNA fragmentation is often necessary prior to library construction or subcloning for DNA sequencing. This protocol describes a method for DNA fragmentation by nebulization, in which the fine mist created by forcing a DNA solution through a small hole in the nebulizer unit is collected. The size of the fragments obtained by nebulization is determined chiefly by the speed at which the DNA solution passes through the hole, altering the pressure of the gas blowing through the nebulizer, the viscosity of the solution, and the temperature. Nebulization is easy, quick, and requires only small amounts of DNA (0.5–5 µg). The resulting DNA fragments are distributed over a narrow range of sizes (700–1330 bp). It requires ligation of DNA before nebulization and end-repair afterward.

RELATED INFORMATION

[Table 1](#) contains a comparison of DNA fragmentation methods.

MATERIALS

Reagents

Agarose gel electrophoresis supplies (see Steps 5 and 8)

Ammonium acetate (2.5 M)

DNA of defined molecular weight (bacteriophage λ DNA or some other large DNA)

DNA sample to be fragmented

Ethanol (100% and 70%)

Glycerol

 TE (pH 7.6)

Equipment

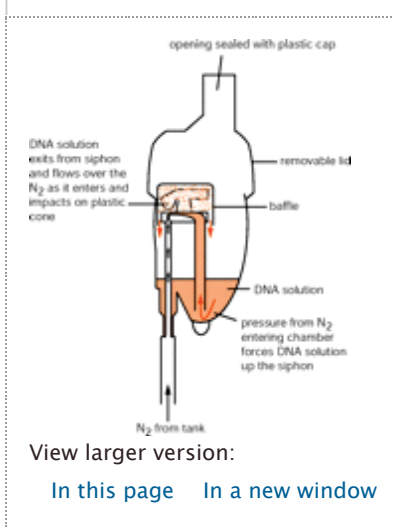
Centrifuge, tabletop

Ice bath

Nalgene tubing

Nebulizer (CIS-US Inc., Bedford, Massachusetts; model number CA-209) (see [Fig. 1](#))

Figure 1.



Nebulizer for random fragmentation of DNA. A DNA solution containing glycerol, for viscosity, is placed in the nebulizer. The nebulizer is attached to a nitrogen gas tank. Pressure from the nitrogen entering the chamber siphons the DNA solution from the bottom of the chamber to the top. The solution exits the siphon and impacts on a small plastic cone suspended near the top of the chamber, thus shearing the DNA.

Nitrogen gas source

QS-T plastic cap

Styrofoam

Tubes, microcentrifuge

METHOD

Modification of the Nebulizer

1. Modify the nebulizer by sealing the mouthpiece hole in the top cover with a QS-T plastic cap. Connect a length of Nalgene tubing to the smaller hole. This tubing will be connected to a source of nitrogen gas.

Calibration of the Nebulizer

2. Prepare TE buffer containing 25% glycerol. Prepare a sample containing 25 µg of bacteriophage λ DNA or other large DNA of defined size in 500 µl of the TE/glycerol solution. Store the DNA solution in an ice bath for 5 minutes.

3. Place the DNA in the cup of a nebulizer connected to a nitrogen gas source and place the nebulizer in an ice-water bath.

Pressure from nitrogen entering the chamber siphons the DNA solution from the bottom of the chamber to the top. The solution exits the siphon and impacts on a small plastic cone suspended near the top of the chamber, thus shearing the DNA.

4. Nebulize the DNA sample at 10 pounds per square inch (psi, 69 KPa) for 90 seconds.

The nebulizer usually leaks a little.

5. After nebulization is completed, collect the DNA by placing the entire unit in a rotor bucket of a bench-top centrifuge fitted with pieces of Styrofoam to cushion it. Centrifuge the nebulizer at 2500 rpm for 30 seconds. Analyze the size of the DNA fragments by electrophoresis through a 1.4% agarose gel (please see [Agarose Gel Electrophoresis](#)) with appropriate standards.

6. If the fragments are too large (>1 kb), repeat the procedure, increasing either the pressure of the nitrogen gas (to 14 psi or 96.5 KPa) or the length of nebulization (2 min). Repeat the procedure until conditions are found that produce a reasonable yield of fragments of the desired size (500 bp to 1 kb).

Nebulization and Recovery of the Target DNA

7. Nebulize the target DNA to produce a reasonable yield of fragments of the desired size (500 bp to 1 kb).
8. Confirm that fragmentation has gone according to plan by analyzing an aliquot of the sample (~200 ng) by electrophoresis through a 1.4% agarose gel as described in Step 5.
9. Distribute the remainder of the sample into two microcentrifuge tubes and recover the DNA by precipitation with 3 volumes of ethanol in the presence of 2.5 M ammonium acetate.
10. Centrifuge the DNA sample at maximum speed for 5 minutes in a microcentrifuge, wash the pellet with 0.5 ml of 70% ethanol at room temperature, and centrifuge again. Remove the ethanol and allow the DNA to dry in the air for a few minutes. Dissolve the pellet of DNA in 25 µl of TE (pH 7.6).