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Generation of DNA fragments by DNase digestion

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William H. Wilson and Declan Schroeder[1]

[1]Manual of Aquatic Viral Ecology

VERVE Net

Declan Schroeder

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Abstract

Generation of DNA fragments by DNase digestion involves digesting DNA for a range of times, then picking the time that gives optimal-sized DNA fragments (typically 1000–4000 bp).

Troubleshooting

1 In a 50 µL reaction volume, resuspend 8 µg DNA in 50 mM Tris·HCl (pH 7.6), 10 mM $MnCl_2$, 100 µg mL^{-1} bovine serum albumin, and 0.01 SU mL^{-1} DNase I.

2 Remove 5 µL aliquots (adding to 45 µL TE buffer, pH 7.6) 0, 0.5, 1, 2, 5, 10, 15, and 30 min after addition of the digestion mixture.

3 Immediately transfer to a tube containing 25 µL Tris-buffered (pH 7.0) phenol.

Note

Typically the shorter incubations, up to 2 min, give optimally sized fragments.

4 Perform a phenol:chloroform (1:1) extraction.

5 Perform a chloroform extraction.

6 Perform a chloroform extraction once more.

7 Precipitate the fragmented DNA.

8 Wash with 70% ethanol.

9 Dry.

10 Resuspend fragmented DNA in 23 µL of Blunt-ending Mix (100 µM dNTPs, 1 × T4 DNA Pol Buffer).

11 Heat at 65°C for 30 min to resuspend DNA and inactivate any DNase I that was carried over.

00:30:00

12 Cool to room temperature.

13 Add 2.5 U Klenow fragment and 5 U T4 DNA polymerase.

14 Incubate the reaction at 37°C for 1 h.
01:00:00

15 The fragmented and blunt-ended virus DNA can be run on a 1% agarose gel prior to excising fragments in the 1000–4000 bp range using a standard gel extraction procedures before downstream cloning.

Note

NB do not excise fragments smaller than 1000 kb, as downstream cloning will preferentially clone the smaller fragments.