DNase Shotgun® Cleavage Kit

Description

DNase Shotgun Cleavage Kit 69281-3

The DNase Shotgun Cleavage Kit is designed for convenient random double stranded cleavage of any DNA. The method is based on the observation that bovine pancreatic DNase I causes double strand scission of DNA in the presence of Mn^{2+} (1,2). Since cleavage is random and can be controlled by varying the enzyme concentration, temperature and/or incubation time, this method is very useful as the initial fragmentation step in the generation of representative libraries having virtually any insert size range.

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Components

- 350µl 10X DNase I Buffer (0.5M Tris-HCl pH 7.5, 0.5mg/ml BSA)
- 350µl 10X $MnCl_2$ (100mM)
- 50U DNase I, ds qualified
- 400µl 6X Stop Buffer (100mM EDTA, 30% glycerol, 0.5% Orange G, 0.075% xylene, cyanol)
- 250µl PCR Markers(50 lanes)

DNase I Cleavage

The following protocol uses a fixed amount of DNA with increasing dilutions of DNase I to find conditions that produce the desired fragment size range. For simplicity we recommend using 10µg of target DNA per reaction, which will produce more than enough fragments for several libraries (on a molar basis, assuming relatively small (<500bp) insert sizes are desired). Because of the amount of target DNA required, it is usually most convenient to use plasmid DNA rather than purified insert DNA. Note that the target DNA must be fairly concentrated (~1.5-5µg/µl) for these reactions to keep the volume small for gel loading purposes. The target DNA must be free of all traces of Mg^{2+}, since this would cause single-strand cleavage by the enzyme. Under these conditions, 1µl of DNase I diluted 1:200 or 1:300 usually gives the highest quantity of 50-150bp fragments in preparation for cloning into the pSCREEN™ T-Vector.

An alternative approach may be more appropriate in cases where these quantities of target DNA are not readily available, with large target DNA that cannot be easily concentrated, or when large fragments are needed (which reduces the number of molecules produced on a molar basis). In these cases the test reactions can be scaled proportionately to find the appropriate enzyme:DNA ratio, and then the appropriate reaction performed again on a larger scale to produce enough material for cloning. When scaling up it is important to maintain the same DNA and enzyme concentrations, as well as ratios, as were used on the trial scale reactions. If larger volumes are used for scaled up reactions, we recommend extracting the reaction twice with phenol:CIAA (1:1) after adding the Stop Buffer. The DNA can then be precipitated with ethanol (as described on p. 2) to concentrate it prior to gel loading.

1. Immediately before use, dilute the DNase I with 1X Buffer + $MnCl_2$ as follows. First make 200µl dilution buffer by adding 20µl 10X DNase I Buffer and 20µl 10X $MnCl_2$ to 160µl sterile deionized H_20. Make a 1:133 dilution by adding 1µl of DNase I to 132µl of this solution. Mix thoroughly by gently finger-flicking the tube (avoid vigorous mixing, since DNase I is susceptible to surface denaturation). Add 30µl of this dilution to a tube containing 15µl buffer to obtain the 1:200 dilution. Repeat the same procedure twice more to obtain 1:300 and 1:450 dilutions.
2. Set up the following reactions (add all components except the enzyme in 0.5ml microcentrifuge tubes at room temperature).

Sample	10X DNase I Buffer	10X $MnCl_2$	DNA	DNase I	H_20
1.	0.9µl	0.9µl	10µg	1µl of 1:133	to 10µl
2.	0.9µl	0.9µl	10µg	1µl of 1:200	to 10µl
3.	0.9µl	0.9µl	10µg	1µl of 1:300	to 10µl
4.	0.9µl	0.9µl	10µg	1µl of 1:450	to 10µl

3. Start the reactions by adding the enzyme and mixing gently. Incubate at room temperature for exactly 10 minutes. Add 2µl (0.2 vol) 6X Stop Buffer to stop the reactions. The Stop Buffer contains 100mM EDTA, 30% glycerol, and tracking dyes.
4. Samples may be analyzed by agarose gel electrophoresis. If small fragments (<1000bp) are desired, 1µl samples should be analyzed on a 2% gel beside the PCR Markers (50, 150, 300, 500, 750, 1000, 1500 and 2000bp). Use 5µl Markers per lane as supplied in loading buffer.

Fractionation of DNA Fragments

The method of fractionation and purification of the reaction products will depend on the fragment size range and personal preference. Any procedure giving good recovery and purity can be used; however, it should be noted that small fragments (<200bp) bind poorly to most commercial resins used for DNA purification. Therefore, we recommend recovering small fragments from agarose gels by electroelution, using low melting point agarose, or using a high-quality agarase that is available from several suppliers. The following protocol has been successfully used in conjunction with electroelution of 50-150bp fragments with the Elutrap (Schleicher and Schuell).

1. Pool cleavage reactions containing fragments in the 50-150bp size range and run on a 2% molecular biology grade agarose gel containing 0.5µg/ml ethidium bromide. Up to 30µg of DNA in this size range can be loaded into a 10mm wide comb in a gel that is 0.75-1cm thick. Run the PCR Markers in an adjacent lane.
2. Excise the band corresponding to 50-150bp fragments and electroelute the DNA following the device manufacturer's instructions.
3. Following elution, extract the sample sequentially with 1 volume TE-buffered phenol, 1 volume of phenol:CIAA (1:1; CIAA = chloroform:isoamyl alcohol 24:1), and 1 volume CIAA. Transfer the final aqueous phase to a fresh tube.
3. Optional: At this point the DNA can be further purified using an Elutip (Schleicher and Schuell) according to the manufacturer's instructions. We observe slightly better results (more efficient cloning) if this step is included.
4. Precipitate the DNA by adding 0.1 volume of 3M sodium acetate and 2 volumes of ethanol. Leave on ice for 30 min. Centrifuge at 12,000 × g for 15 min. Drain carefully, rinse the pellet with 70% ethanol, dry, and resuspend DNA in 30µl TE (10mM Tris-HCl pH 8.0, 1mM EDTA). Determine the DNA concentration by reading the A_{260} of a 3µl sample in 300µl water ($1A_{260}$ unit = 50µg/ml). Expect an overall recovery of about 20%, or 6µg DNA if starting with 30µg. If the average fragment size is 100bp, 6µg corresponds to about 100pmol of DNA, or 200pmol ends. The DNA is now ready for further treatment, such as Single dA™ Tailing, in preparation for cloning.

References

1. Campbell, V.W. and Jackson, D.A. (1980) *J. Biol. Chem.* **255**, 3726-3735.
2. Anderson, S. (1981) *Nucleic Acids Res.* **9**, 3015-3027.