SAMPLE CONCENTRATOR Application note: A04-001B

Concentration of DNA samples using the Techne sample concentrator

Introduction

The Techne sample concentrator uses the principle of solvent evaporation to concentrate samples. Simply heating the solution in a Dri-Block® heater does not greatly increase the evaporation rate. However when this is combined with a stream of inert gas (usually nitrogen) directed onto the surface of the solution, vapour is rapidly driven off and the sample is quickly concentrated. A large number of samples can be processed in a matter of minutes, where other methods such as freeze drying and vacuum centrifugation can take several hours.

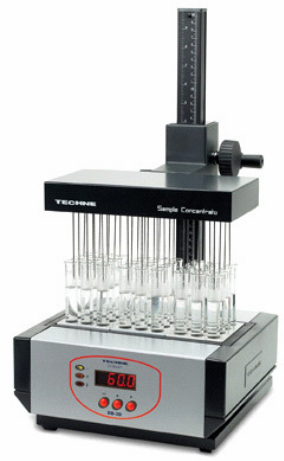

Figure 1: The Techne sample concentrator with a DB-3D Dri-Block® heater.

The nitrogen required for the sample concentrator can be supplied using a traditional gas cylinder fitted with an appropriate regulator to supply the requisite flow rate (maximum 15 litres/minute at ≤2psi). Gas cylinders are however difficult to handle due to their size and weight, need to be replaced on a regular basis and are subject to a number of guidelines to ensure their safe use[1]. Alternatively a nitrogen generator* with a suitable nitrogen output can be used. Since the sample concentrator generally only requires only a small proportion of the output of a nitrogen generator, the purity of the nitrogen produced is typically >99%.

There are numerous applications for the sample concentrator in many scientific areas including molecular biology, drug screening and analytical procedures. In this application note we compare three methods for concentration of DNA solutions: using the sample concentrator to dry the sample and the traditional methods of ethanol and 2-propanol precipitation. We also look at the time taken to evaporate samples of different ethanol concentration.

Methods

Calf thymus DNA (Sigma D3664) was diluted in molecular biology grade water to give solutions of nominal concentration 50, 25, 10 and 5µg/ml (samples labelled sample 1 to 4 respectively). The absorbance of each solution at 260 and 280nm was read in a Jenway Genova spectrophotometer using the A_{260}/A_{280} mode. Nine 0.5ml aliquots of each concentration of DNA were placed in 1.5ml microfuge tubes. Triplicate samples at each concentration were used for (a) sample concentration, (b) ethanol precipitation and (c) 2-propanol precipitation.

For sample concentration, the tubes were placed in the Dri-Block® heater, set at 50ºC. Needles were inserted into the matrix head of the sample concentrator at the required positions. The sample concentrator was connected to a nitrogen generator* set at 7 litres per minute flow rate. The needles were lowered into the tops of the tubes to about 10-20mm above the surface of the meniscus and the samples incubated until they were just dry. The tubes were removed and 0.5ml water added to each. The DNA was allowed to re-dissolve for at least an hour at room temperature before the absorbance at 260 and 280nm was re-measured.

For ethanol precipitation, 0.1 volume (50µl) of 3M sodium acetate, pH 5.2 (Sigma S7899) was added to each tube followed by 2 volumes (1.1ml) of cold ethanol (Sigma E7023). The tubes were mixed and stored at -20ºC for at least 30 minutes. The tubes were then centrifuged for 10 minutes, the supernatant decanted and the pellets washed with 1ml of 70% ethanol. The tubes were then centrifuged briefly, the supernatant decanted and the pellets allowed to air dry. 0.5ml water was added to each tube and the DNA was allowed to re-dissolve overnight at 4ºC. The absorbance at 260 and 280nm was then re-measured.

For 2-propanol precipitation, 0.1 volume (50µl) of 3M sodium acetate, pH 5.2 was added to each tube followed by 1 volume (550µl) of 2-propanol (Sigma I9516). The samples were then treated as above for ethanol precipitation.

To test the time taken to evaporate a volume of solution using the sample concentrator, six solutions were prepared containing 100, 80, 60, 40, 20 and 0% ethanol in water. 10ml of each were placed in 15ml centrifuge tubes with 0.1ml graduations and placed in the Dri-Block® heater set at 50ºC. Needles were inserted into the matrix head of the sample concentrator at the required positions. The sample concentrator was connected to a nitrogen generator* set at 7 litres per minute flow rate. The needles were lowered into the tops of the tubes to about 10-20mm above the surface of the meniscus. At regular time intervals the volume of each sample was noted. The needles were lowered into the tubes as the volume of sample reduced to maintain the optimum flow of nitrogen on the surface of the sample. The experiment was then repeated without the use of the sample concentrator, using the Dri-Block® heater only, set at 50ºC.

Results

DNA and RNA are often precipitated with ethanol or 2-propanol to either concentrate the sample or to change the reaction buffer. This can be time consuming if many samples need to be processed and there is a risk of loss of sample or introduction of contaminants. For this experiment, 0.5ml volumes of DNA solutions of various concentration were either concentrated to dryness or alcohol precipitated then resuspended in the same volume to determine whether there was any loss of sample in each process. The results are presented in Figure 2.

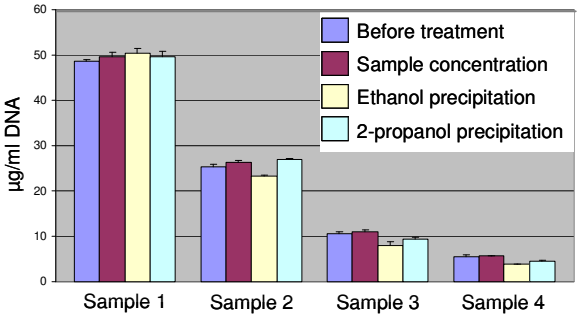

Figure 2: DNA concentration of each sample calculated from the A_{260} and A_{280} absorbance values. Values are the means of triplicate samples.

The graph in Figure 2 indicates that none of the DNA in the samples at the two highest concentrations (50 and 25µg/ml, samples 1 and 2 respectively) were lost by any of the methods of treatment. However at lower DNA concentrations, a significant proportion (up to 30%) of the sample was lost when alcohol precipitated, either due to incomplete precipitation or to loss of the sample with the supernatant. Table 1 gives the values for the 10 and 5µg/ml samples (samples 3 and 4).

	Sample 3 (% loss)	**Sample 4 (% loss)**
Sample concentration	-4.8 ± 2.5	-2.9 ± 1.3
Ethanol precipitation	23.9 ± 6.3	31.2 ± 1.6
2-propanol precipitation	10.5 ± 2.8	19.5 ± 4.3

Table 1: Percentage loss of DNA from samples 3 and 4 after resuspension following sample concentration or alcohol precipitation.

In addition to the loss of some of the sample during precipitation, the A_{260}/A_{280} ratios of samples which were alcohol precipitated were significantly reduced compared to the samples dried in the sample concentrator. This would suggest that there was some carry over of alcohol or salts from the precipitated samples.

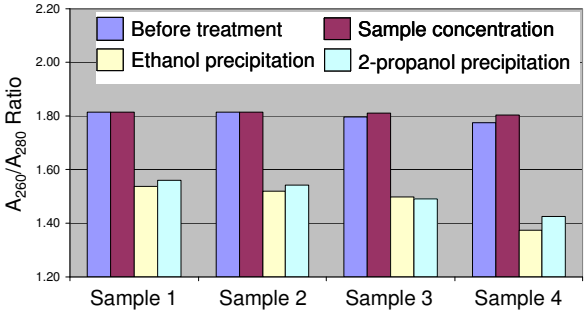

Figure 3: A_{260}/A_{280} ratio of each sample. Values are the means of triplicate samples.

It is sometimes necessary to concentrate or dry larger volumes of solution prior to down-stream processing. In the second experiment, solutions containing different proportions of ethanol were placed in the sample concentrator and the rate of evaporation measured. The results are presented in Figure 4.

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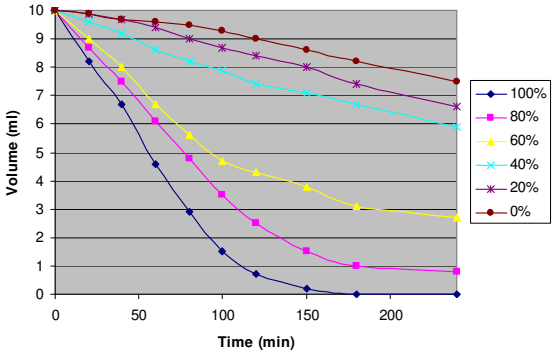

Figure 4: Rate of evaporation of ethanol solutions using the sample concentrator. % values refer to the % ethanol by volume.

As would be expected, 100% ethanol was the quickest to evaporate as it is the most volatile of the solutions tested. Complete evaporation of 10ml solution at a Dri-Block® temperature of 50ºC, in 15ml tubes took less than 3 hours. Pure water was reduced to approximately 75% of the original volume when the experiment was stopped after 4 hours. In comparison, in the same experiment performed without using the sample concentrator, using the Dri-Block® heater only, 100% ethanol was reduced by only 20% after 30 hours.

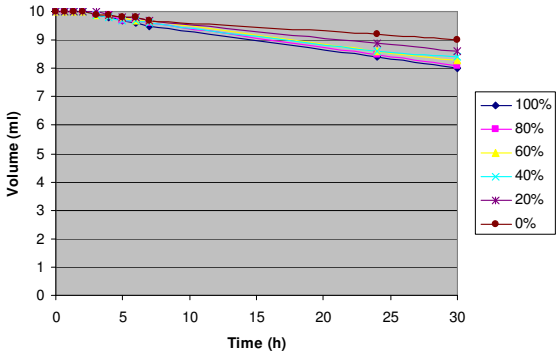

Figure 5: Rate of evaporation of ethanol solutions using the Dri-Block® heater only, without the sample concentrator.

Conclusions

We have demonstrated that use of the sample concentrator in conjunction with a nitrogen generator* to remove the solvent from DNA samples, is a relatively rapid process which resulted in no loss of sample quality or quantity. The time taken to evaporate 0.5ml samples dissolved in water was 70-90 minutes with very little hands-on time compared to at least 2-3 hours for alcohol precipitation, depending on sample numbers. Use of the sample concentrator is therefore ideal for concentrating medium to small sample volumes where buffer exchange or removal of salts is not required.

References

1. Safe use of gas cylinders. HSE (June 2004). http://www.hse.gov.uk/cdg/pdf/safusgc.pdf

* Results cannot be guaranteed with any other nitrogen generator. For further details please contact technehelp@bibby-scientific.com.