Promega Notes Magazine **Number 59, 1996, p. 46**

BSA and Restriction Enzyme Digestions

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Recently we have developed performance assays comparing the effect of the addition of acetylated bovine serum albumin (BSA) to restriction enzyme digestions. We compared the effects of adding BSA to restriction enzyme digests performed using enzymes from several different suppliers, and using DNA purified using two different techniques. We have also examined the effect of the presence of BSA on restriction enzyme digestions performed using different reaction vessels. In nearly all instances, the presence of BSA enhanced the performance of restriction enzymes by at least two-fold.

Introduction

Restriction enzymes have been a trusted tool of the molecular biologist for more than fifteen years. Researchers rely on the specificity of these enzymes to cut at distinct sites within a particular template DNA. Many factors affect the performance of restriction enzymes, including template purity, reaction buffer components, temperature, time and each enzyme's unique kinetics.

Through years of producing high quality restriction enzymes, we have developed application-based assays which ensure product purity, consistency and performance. Recently, we collected performance data comparing a variety of restriction enzymes and using several DNA purification methods, reaction vessels and suppliers, and found that in nearly all cases, enzyme performance is enhanced by the presence of acetylated bovine serum albumin (BSA) in the reaction. The BSA used in all Promega assays and products is acetylated using acetic anhydride and is extensively tested to ensure that it is free of contaminating endonuclease and exonuclease activities. Where referred to here, BSA always means acetylated bovine serum albumin.

Users of restriction enzymes often add excess enzyme to their reaction, preferring the ease of pipetting 1µl to the more complex dilution of the enzyme. Excess enzyme often serves the same function as BSA, providing additional protein which stabilizes the enzyme and balances any negative effects arising as a result of enzyme interaction with solid surfaces and/or the air-liquid interface. At times, however, the enzyme is not in excess, or its activity is reduced due to nonoptimal reaction conditions. In these cases, the positive effect of BSA addition is most pronounced.

Our results have led us to begin including BSA in all of our restriction enzyme products, at no additional charge to our customers. This article briefly reviews examples of our performance testing.

DNA purification methods

In this analysis, DNA purified using Promega's Wizard® *Plus* Minipreps DNA Purification System was compared with DNA purified using a miniprep DNA purification system from another major supplier (Supplier A). Regardless of which system was used, the addition of BSA enhanced the performance of the restriction enzymes tested.

In the example shown in Figure 1, the pGEM®*-3Zf(+) Vector was purified using either Wizard® *Plus* Minipreps (Figure 1, Panels A and B) or Supplier A minipreps (Figure 1, Panels C and D). Restriction enzyme digestion was performed using the enzyme *Hin*d III. Each reaction was performed either with 0.1mg/ml BSA (Figure 1, Panels A and C) or without added BSA (Figure 1, Panels B and D). The addition of BSA improved the enzyme's performance, reducing the amount of enzyme required to completely digest the template from 6 units (Figure 1, Panels B and D) to 2 units (Figure 1, Panels A and C).

**U.S. Pat. No. 4,766,072 has been issued to Promega Corporation for transcription vectors having two different bacteriophage RNA polymerase promoter sequences separated by a series of unique restriction sites into which foreign DNA can be inserted.*

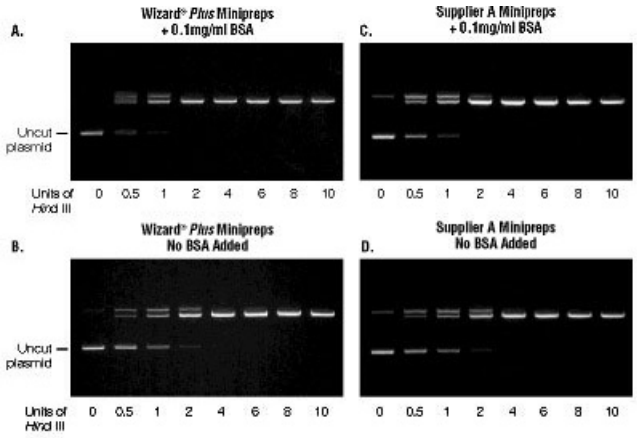

Figure 1. Comparison of the effect of DNA purification methods on the amount of restriction enzyme required for complete digestion of the pGEM®-3Zf(+) Vector. The pGEM®-3Zf(+) Vector was purified using either Wizard® *Plus* Minipreps DNA Purification System (Panels A and B) or Supplier A miniprep protocols (Panels C and D). The *Hin*d III was diluted to 1u/µl in 1X assay buffer with or without 0.5mg/ml BSA and used immediately. The plasmid was digested with 0, 0.5, 1, 2, 4, 6, 8 or 10 units of *Hin*d III for 1 hour at 37°C. **Panels A and B:** Plasmid purified using Wizard® *Plus* Minipreps. *Hin*d III digestion with 0.1mg/ml BSA (Panel A) and without BSA (Panel B). Complete digestion is achieved with 2 units in Panel A, but requires 6 units in Panel B. **Panels C and D:** Plasmid purified using supplier A plasmid purification system. *Hin*d III digestion with 0.1mg/ml BSA (Panel C) and without BSA (Panel D). Complete digestion is achieved with 2 units in Panel C, but requires 6 units in Panel D.

Reaction vessels

To determine the effect of the presence of BSA on restriction enzyme digestions in different reaction vessels, we compared three different vessels: 1) standard 0.5ml Eppendorf® polypropylene microcentrifuge tubes; 2) "low-binding" 96 well microtiter plates (Costar® Ultra Low Cluster, Cat.# 3474); and 3) standard polystyrene 96 well microtiter plates (Dynatech Immulon 1, Cat.# 011-101-3555).

pGEM®-3Zf(+) Vector DNA was digested with the enzyme *Eco*R I. Each restriction enzyme digestion was performed both with and without the addition of 0.1mg/ml BSA, in each of three different reaction vessels (Figure 2). The addition of BSA improved the enzyme's performance in the reaction with the "low-binding" plate (lanes 4 and 5), but the added BSA was unable to compensate for the stronger binding of a standard 96 well plate (lanes 6 and 7). It is likely that the BSA serves to block binding of the restriction enzyme to the walls of reaction vessels.

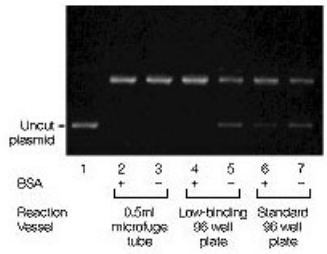

Figure 2: Comparison of reaction vessels using Promega *Eco*R I, with and without BSA. The pGEM®-3Zf(+) Vector was purified using Wizard® *Plus* Minipreps and digested using *Eco*R I. The *Eco*R I was diluted to 1u/µl in 1X assay buffer with or without 0.5mg/ml BSA and used immediately. The amount of *Eco*R I used in each digestion was 2 units. Tubes were standard 0.5ml polypropylene Eppendorf® microfuge tubes. Low-binding plates were 96 well Costar® Ultra Low Cluster (Cat.# 3474). Standard plates were 96 well Dynatech Immulon 1 (Cat.# 011-101-3555). Lane 1: uncut plasmid; Lanes 2 and 3, 4 and 5, 6 and 7: tubes, low-binding, and standard plates, with and without BSA, respectively.

Comparison of restriction enzymes from various suppliers

Figure 3 shows the results of a comparison of the effect of the addition of BSA to restriction enzyme digestions performed using enzymes from five different suppliers: Promega and Suppliers A, B, C and D.

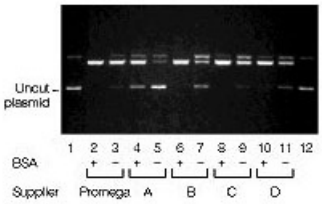

Figure 3: Vendor comparison of plasmid restriction using *Cla* I, with and without BSA. The pGEM®-7Zf(+) Vector was prepared using Wizard® *Plus* Minipreps. The *Cla* I was diluted to 1u/µl in 1X assay buffer with or without 0.5mg/ml BSA and used immediately. The amount of *Cla* I used in each digestion was 0.5 units. Lanes 1 and 12: uncut plasmid; Lanes 2 and 3: Promega *Cla* I with and without BSA respectively; Lanes 4 and 5, 6 and 7, 8 and 9, 10 and 11: Suppliers A, B, C and D, with and without BSA, respectively.

pGEM®-7Zf(+) Vector DNA was digested using the enzyme *Cla* I manufactured by each of the 5 different suppliers. Each reaction was performed with and without the addition of BSA to a final concentration of 0.1mg/ml. In the majority of cases, the addition of BSA improved the enzyme's performance. These results, and the results of identical experiments using the enzymes *Sph* I and *Not* I, are summarized in Table 1.

Table 1. Supplier Comparison Summary: Increase in Units of Restriction Enzyme Necessary to Achieve Complete Plasmid Digestion Without the Addition of BSA.

Restriction Enzyme	Supplier	pGEM® Vector	Increase in Units of Enzyme[2]
Sph I	Promega[1]	3Zf(+)	5X
	A	3Zf(+)	32X
	B	3Zf(+)	2X
	C	3Zf(+)	32X
	D[1]	3Zf(+)	6X
Not I	Promega[1]	11Zf(+)	2X
	A[1]	11Zf(+)	>1X
	B	11Zf(+)	2X
	C	11Zf(+)	5X
	D	11Zf(+)	>1X
Cla I	Promega[1]	7Zf(+)	6X
	A[1]	7Zf(+)	4X
	B	7Zf(+)	6X
	C	7Zf(+)	3X
	D[1]	7Zf(+)	2X

[1]*BSA recommended by vendor*

[2]*Increase in units of enzyme required for complete digestion if BSA* ***is not added*** *to the reaction.*

Summary

Restriction enzymes are used in a vast array of applications and reaction conditions. Our performance testing has conclusively shown that, under certain conditions, many restriction enzymes show enhanced activity with the addition of BSA to the digestion reaction. No negative effects have been associated with the presence of BSA in restriction enzyme digestions. We therefore recommend the addition of BSA to a final concentration of 0.1mg/ml in all restriction enzyme digestion reactions. When performing restriction enzyme dilutions prior to setting up digestion reactions, we recommend the use of 1X assay buffer containing 0.5mg/ml BSA. In cases where the addition of BSA is not desired, performance of Promega's restriction enzymes is sufficient to perform complete digestion, but a moderate increase in the amount of enzyme added to the reaction may be required. BSA is now included with all Promega restriction enzymes.
