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Removal of DNA from lysates of *E. coli* cells and French bean tissue prior to electrophoresis.

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Introduction

Routine preparation of cell lysates for electrophoresis and for Western blotting require pre-treatment of the sample to prevent the high molecular weight DNA from interfering with protein separation on SDS PAGE gels. Most commonly, this procedure involves shearing the DNA by passing the sample through a small gauge needle. However, this is a time consuming process which is difficult to reproduce and which often does not completely solve the problem of samples being too viscous to enter the gel efficiently.

Enzymatic digestion of the DNA with DNase would be another option to shearing contaminating DNA. This method has the disadvantage of contaminating the sample with an additional protein and the risk of degrading the proteins of interest during the mandatory enzymatic incubation at 37°C.

In our experiments, we have employed the Vivascience DNA Removal Kit to effectively remove DNA from two different lysates without loss or degradation of the proteins. Samples were then analyzed on SDS PAGE gels.

1. Material and Methods

The two samples were prepared as follows:

A sample from French bean was prepared by germinating a set of seeds. After germination, a 5 g sample of these seeds with seed coats removed, were cut into fine slices, mixed with 20 ml DNA binding buffer A-D from the Vivascience DNA removal kit and then homogenized using a Potter-Elvehjem homogenizer. Finally, the lysate was clarified through a 0.8 mm Maxi Vivascience clarification filter at 4000 x g for 5 minutes, which is also a kit component. In addition, 10 mg of *E. coli* cells were homogenized as above after being vortexed with 10 ml DNA binding buffer A-D followed by clarification as above.

For DNA removal, two Mini Vivapure D M spin columns were equilibrated with 400 µl DNA binding buffer A-D. 400 µl of each sample was spun through an equilibrated spin column at 4000 x g for 5 minutes. The flow-through was collected and stored at 4°C for further analysis by gel electrophoresis. The columns where washed with 400 µl of DNA Binding Buffer A-D to remove any traces of protein still bound to the membrane and centrifuged at 4000 x g for 5 minutes.

This flow-through was also collected and stored. Bound DNA was eluted from the membrane in a final step by washing the spin columns with 400 µl of DNA elution buffer B-D and spinning at 4000 x g for 5 minutes. This flow-through was again collected and stored. Samples where diluted 1:3 with Laemmli sample buffer containing β-mercaptoethanol and loaded onto 10% Tris-HCl pre cast gels from Bio-Rad in TGS buffer after denaturation at 95°C for 5 minutes. For DNA gels, samples were diluted 1:2 in TBE sample buffer and separated on 4-20% TBE Bio-Rad pre cast gels in TBE buffer after denaturation at 70°C for 3 minutes.

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2. Results

All collected samples were analyzed by DNA and protein gel electrophoresis as described in Materials and Methods. Gel 1 shows the separation of a protein extract from germinated French bean seeds. As can be seen in the gel, the starting material and the flow-through have the same protein band pattern. This would indicate, that little or no protein are lost in the process.

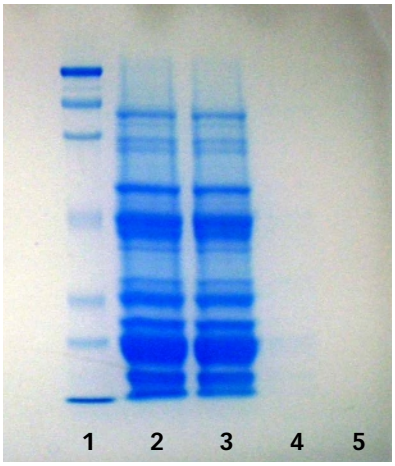
Neither the wash fraction after DNA removal nor the fraction after DNA elution contained detectable amounts of proteins as shown in the Coomassie stained SDS gel (Gel 1.) Gel 2 shows the same experiment run with an *E. coli* extract. This gel was silver stained for more sensitive protein detection.

The wash-fraction, which was employed to elute any traces of proteins still bound to the membrane after DNA removal only contains very weak protein bands, showing that nearly all proteins in the lysate pass the membrane on the first passage. No visible protein bands could be detected in the DNA elution fraction even using this very sensitive staining method.

In summary, it can be said that no protein is lost in the process of DNA removal with the Vivascience DNA removal kit.

Gel 1, Protein gel of French bean tissue

Gel 1 French bean tissue samples before and after DNA removal, 10% Tris-HCl pre cast gels from Bio-Rad in TGS buffer, Coommassie blue stained.



Lane 1 = Marker

Lane 2 = French bean tissue (start material)

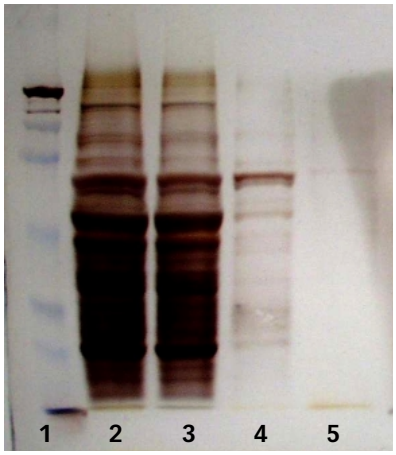
Lane 3 = French bean Vivapure D M flow-through

Lane 4 = French bean Vivapure D M wash

Lane 5 = French bean Vivapure D M elution

Gel 2, Protein gel of *E. coli*

Gel 2, *E. coli* lysate samples before and after DNA removal, 10% Tris-HCl pre cast gels from Bio-Rad in TGS buffer, Silver stained



Lane 1 = Marker

Lane 2 = *E. coli* start material

Lane 3 = *E. coli* Vivapure D M flow-through

Lane 4 = *E. coli* Vivapure D M wash

Lane 5 = *E. coli* Vivapure D M elution

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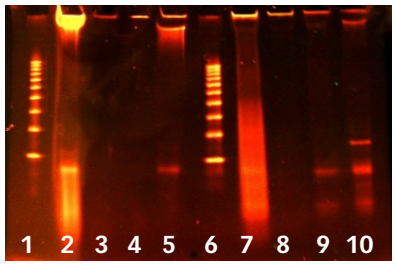
Analyzing the samples on a DNA gel demonstrates the efficient DNA removal. While the extracts contain clearly visible amounts of long and short DNA fragments, the flow-through fractions after DNA removal show no detectable amounts of DNA. DNA was eluted from the membrane if necessary using the elution buffer B-D from the kit.

Gel 3, DNA of *E. coli* and French bean DNA gel.

Gel 3, *E. coli* lysate and French bean tissue samples before and after DNA removal, 20% TBE Bio-Rad pre cast gels in TBE buffer, Ethidium bromide stained Gel 3 stained with Ethidium bromide



Lane 1 = Marker

Lane 2= *E. coli* start material

Lane 3= *E. coli* Vivapure D M flow-through

Lane 4= *E. coli* Vivapure D M wash

Lane 5= *E. coli* Vivapure D M elution

Lane 6= Marker

Lane 7= French bean start material

Lane 8= French bean Vivapure D M flow-through

Lane 9= French bean Vivapure D M wash

Lane 10= French bean Vivapure D M elution

3. Discussion and Conclusion

In this experiment, we could demonstrate that DNA contained in samples from *E. coli* and French bean extracts can be completely removed using the the Vivapure DNA Removal Kit while maintaining the original protein content.