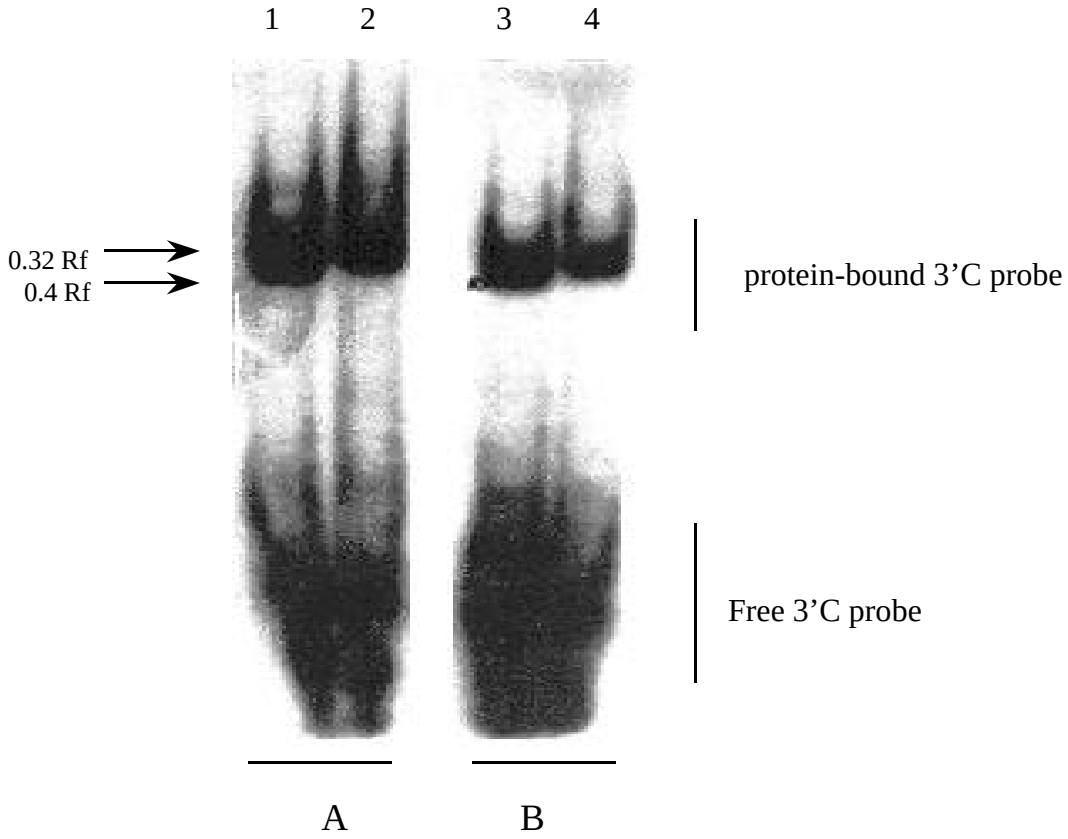


**Figure 3.1.** Gel shift assay to test the activity of purified DdRPA from undifferentiated and differentiated cells that binds to 3'C probe. Lanes 1 and 3 correspond to undifferentiated DdRPA(0.4 Rf) Lanes 2 and 4 correspond to differentiated DdRPA (0.32Rf). The 3' C probe was used as probe. In A, the probe used was 1/1 (explained in material methods) and in B the probe was diluted 1/10.

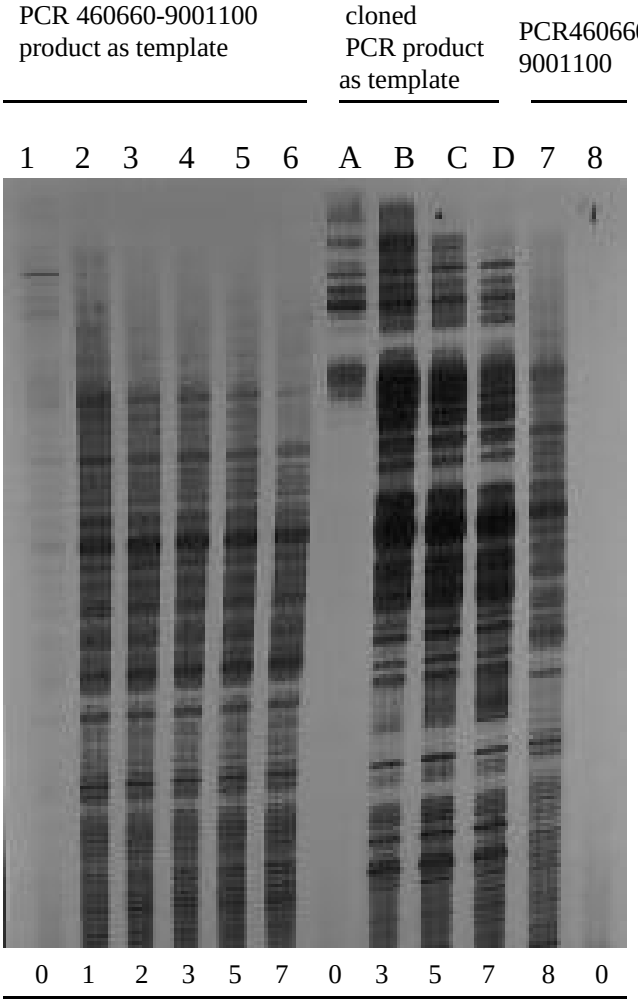


**Figure 3.2.** Test of DNase I digestion times on the PCR460660-9001100 template and its cloned counterpart using primer extension footprinting. 5ul 1:100 DNase I was applied for each lane except lanes 1,8 and A. The time of digestion for each sample is designated at the bottom of the figure. 9001100 primer was used.

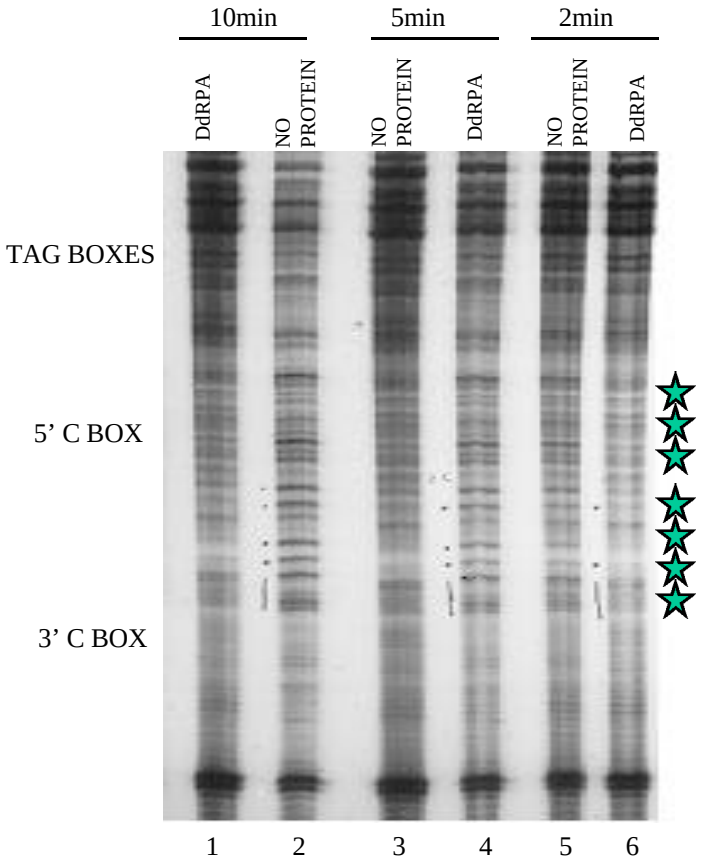


**Figure 3.3.** DNase I footprint assay to test the effect of digestion times on template with and without the addition of protein. DdRPA from undifferentiated cells was assayed using the endlabeling footprinting technique. 460660K-9001100 was used as the template, poly dI:dC was used as the nonspecific competitor. The C and TAG boxes are shown. Stars indicate regions of protection. DNase I digestion time (2, 5 and 10 min) is indicated on top.

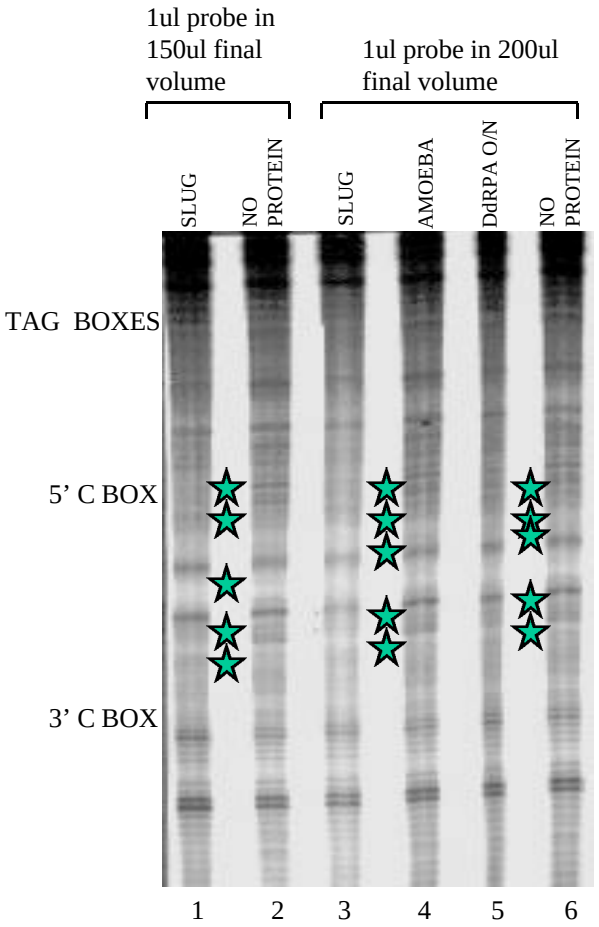


**Figure 3.4.** DNase I footprint assay to determine the effects of extract concentration. The extracts used were DdRPA from differentiated cells (O/N), ameoba and slug extracts. The endlabeling footprinting technique was used. 460660K-9001100 was used as template, poly dA:dT was used as nonspecific competitor. DNase I digestion time was 5 minutes for each lane. The C and TAG boxes are shown. Footprints are indicated by stars.

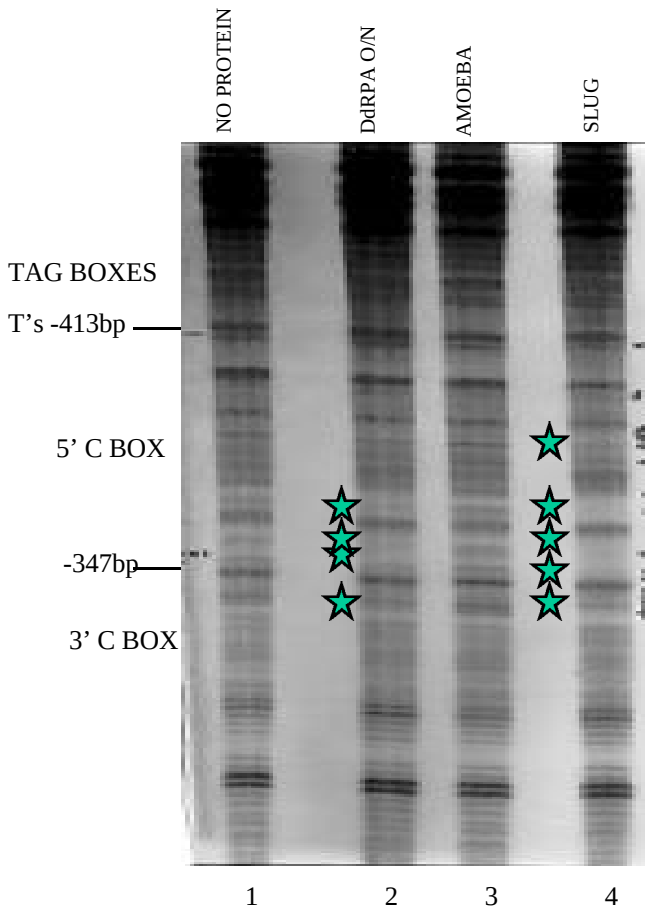


**Figure 3.5.** DNase I footprint assay to compare patterns of protection generated by DdRPA from differentiated cells (O/N), ameoba and slug extracts. The endlabeling footprinting technique was used. 460660K-9001100 was used as template, poly dI:dC was used as nonspecific competitor. DNase I digestion time was 5 minutes for each lane. The C and TAG boxes are shown. Footprints are indicated by stars.

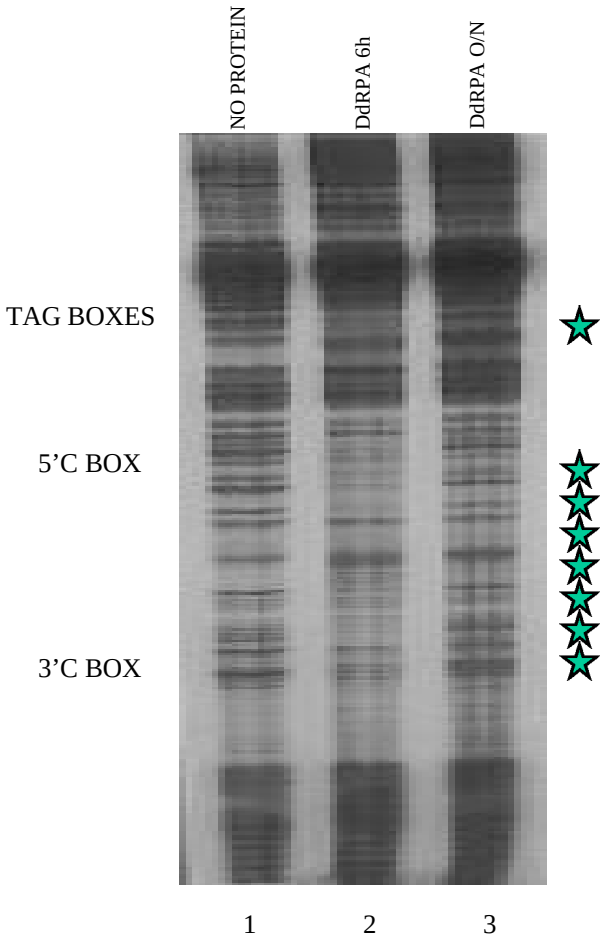


**Figure 3.6.** DNase I footprint assay to compare patterns of protection generated by DdRPA from differentiated (O/N) and undifferentiated (6h) cells over a large region of the promoter. The endlabeling footprinting technique was used. 460660K-9001100 was used as template, poly dI:dC was used as nonspecific competitor. DNase I digestion time was 5 minutes for each lane. The C and TAG boxes are shown. Footprints are indicated by stars.

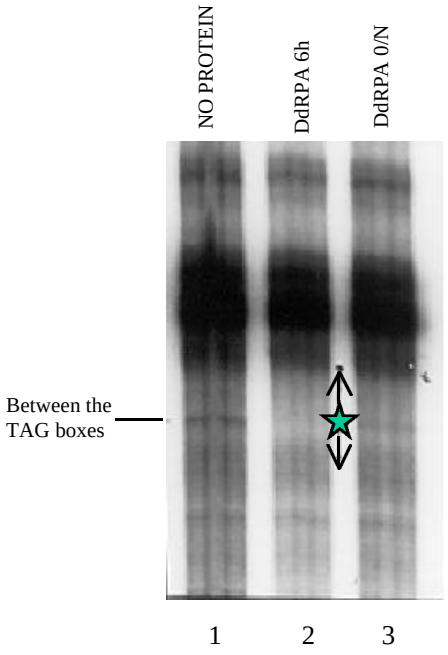


**Figure 3.7.** DNase I footprint assay comparing protected regions over the TAG box region generated by DdRPA from differentiated (O/N) and undifferentiated (6h) cells. The endlabeling footprinting technique was used. N-9001100K was used as template, poly dI:dC was used as nonspecific competitor. DNase I digestion time was 5 minutes for each lane. The TAG boxes are shown. Footprints are indicated by the star.

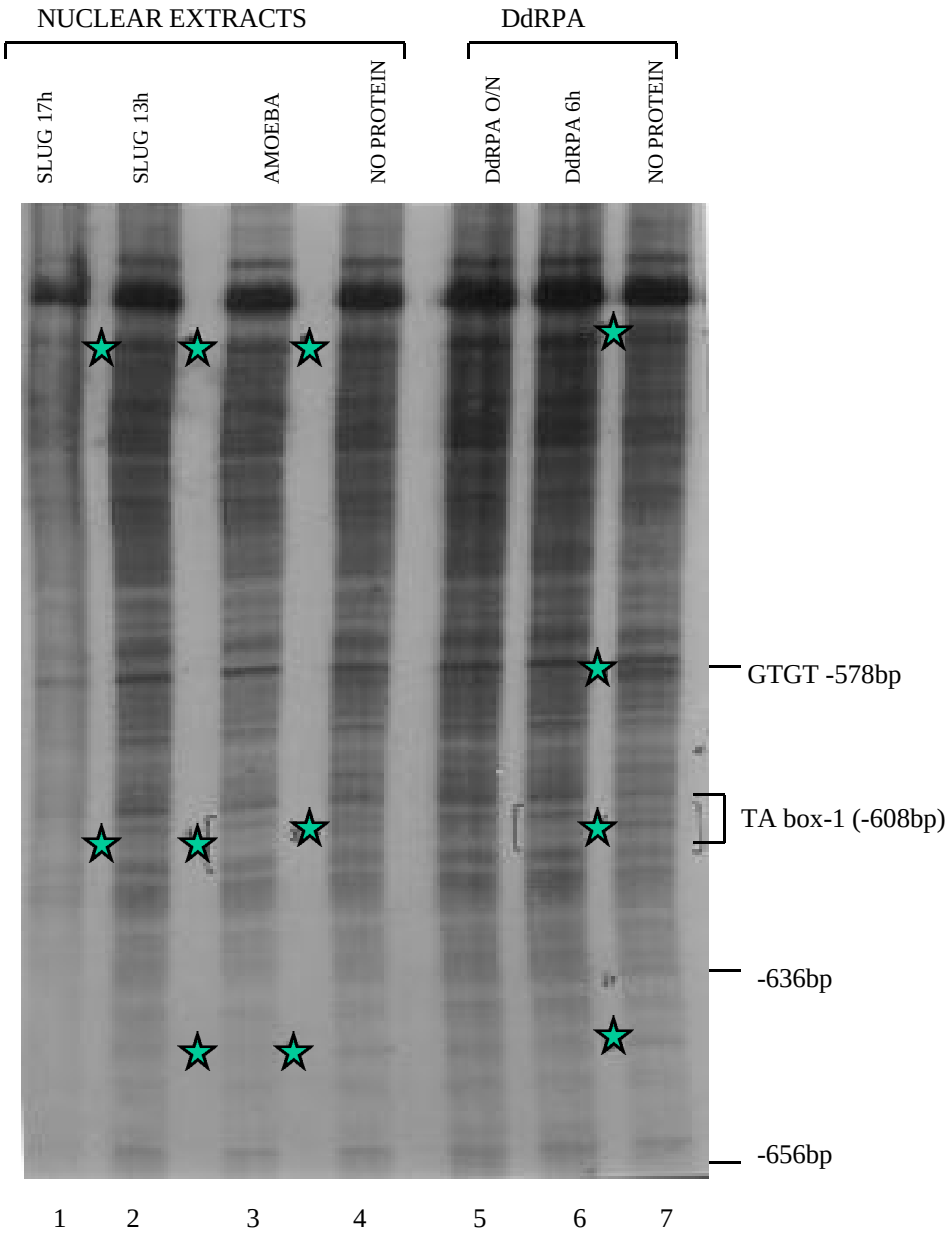


**Figure 3.8.** DNase I footprint assay to compare extracts from DdRPA of differentiated (O/N) and undifferentiated (6h) cells, ameoba, slug (13h) and slug(17h). The endlabeling footprinting technique was used. 9001100K-460660 was used as template, poly dA:dT was used as nonspecific competitor. DNase I digestion time was 5 minutes for each lane. TA box-1 and other sequences are shown.. Footprints are indicated by stars.

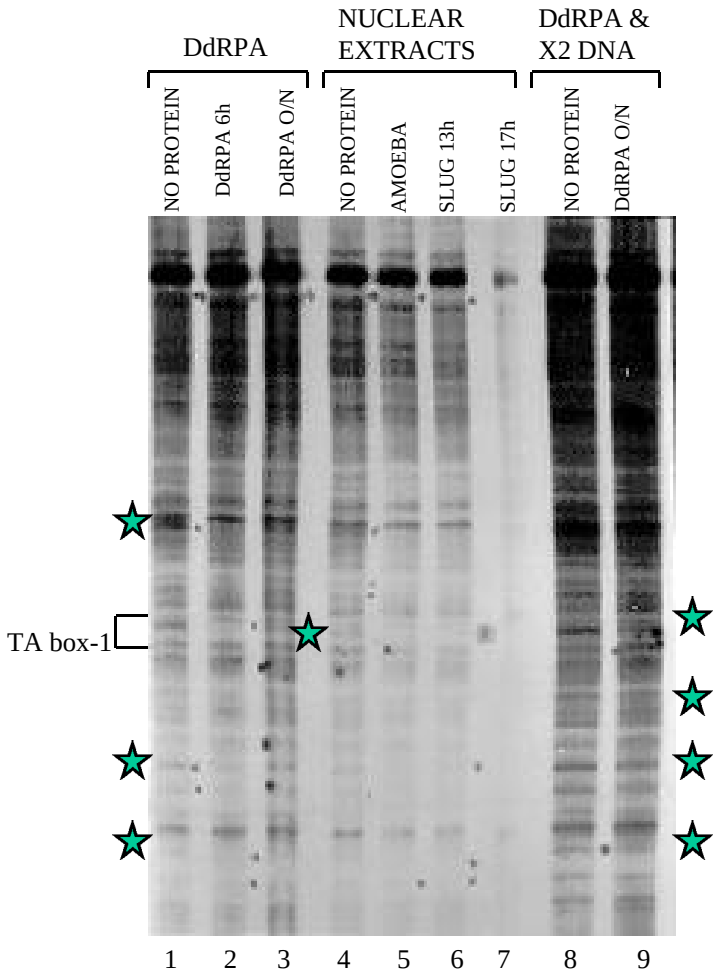


**Figure 3.9.** DNase I footprint assay of DdRPA from differentiated (O/N) and undifferentiated (6h) cells, ameoba, slug (13h) and slug (17h) extracts using the endlabeling footprinting technique. 9001100K-460660 was used as template, poly dA:dT was used as nonspecific competitor. The amount of probe was double in lanes 8 and 9. DNase I digestion time was 5 minutes for each lane. The location of TA box-1 is indicated on the left. Footprints are indicated by stars.

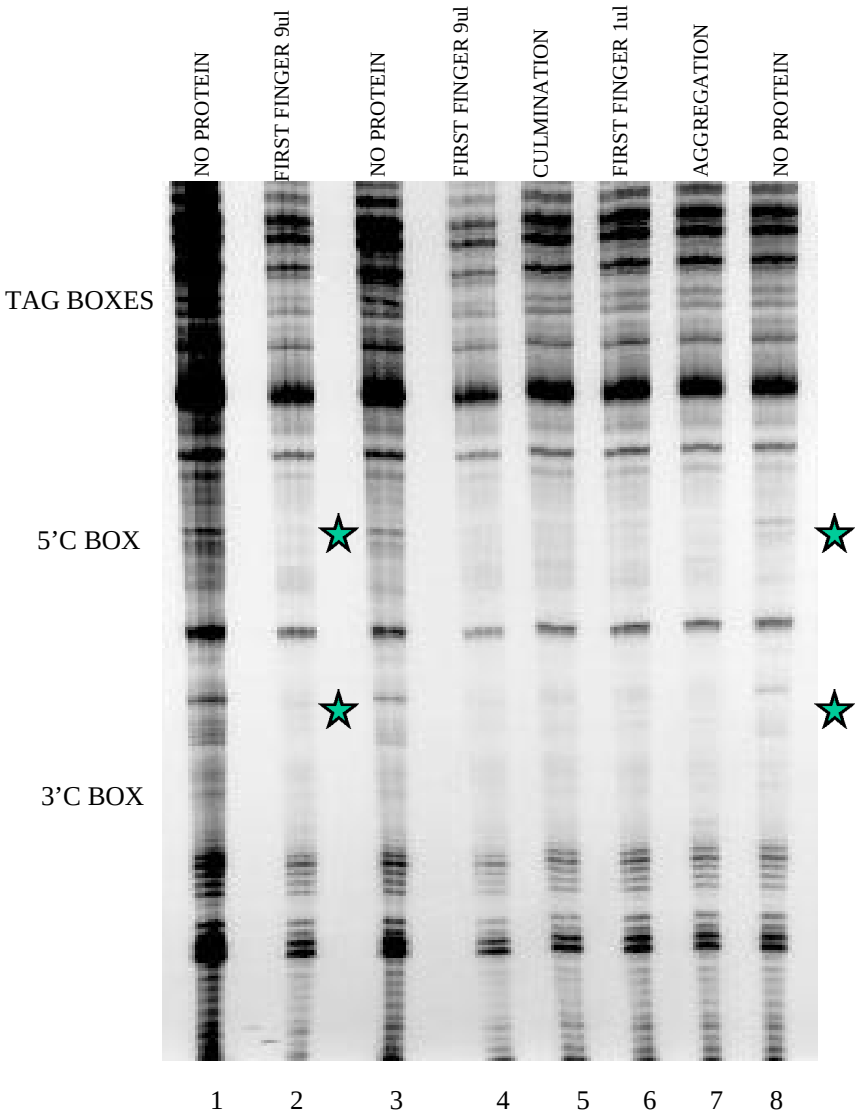


**Figure 3.10.** DNase I footprint assay of aggregation (8h), first finger (16h) and culmination (21-22h) extracts using the endlabeling footprinting technique. The volume of extract used is indicated near the lane label. 460660K-9001100 was used as template, poly dI:dC was used as nonspecific competitor. DNase I digestion time was 5 minutes for each lane. The C and TAG boxes are shown. Footprints are indicated by stars.

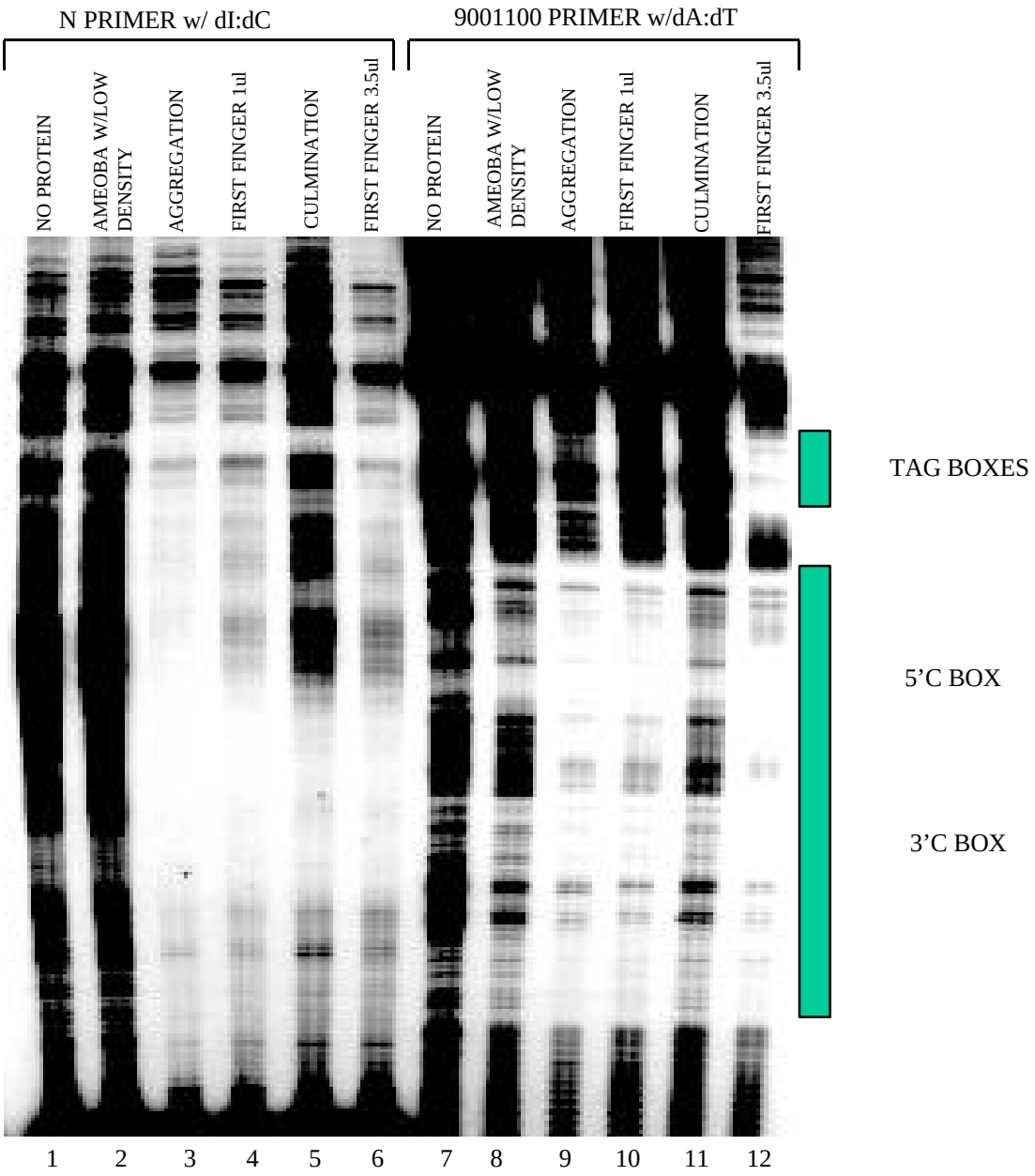


**Figure 3.11.** DNase I footprint assay to detect developmentally regulated footprints. Extracts from ameoba grown at low density, aggregation(8h), first finger(16h) and culmination (21-22h) were tested using the primer extension footprinting footprinting technique. p2124 was used as template, N and 9001100 were applied as primers. Poly dI:dC with N primer and poly dA:dT with 901100 primer were used as nonspecific competitors. DNase I digestion time was 8 minutes for lanes 1 and 7, whereas it was 6 min for other lanes. The C and TAG boxes are shown. Footprints are indicated by bars.

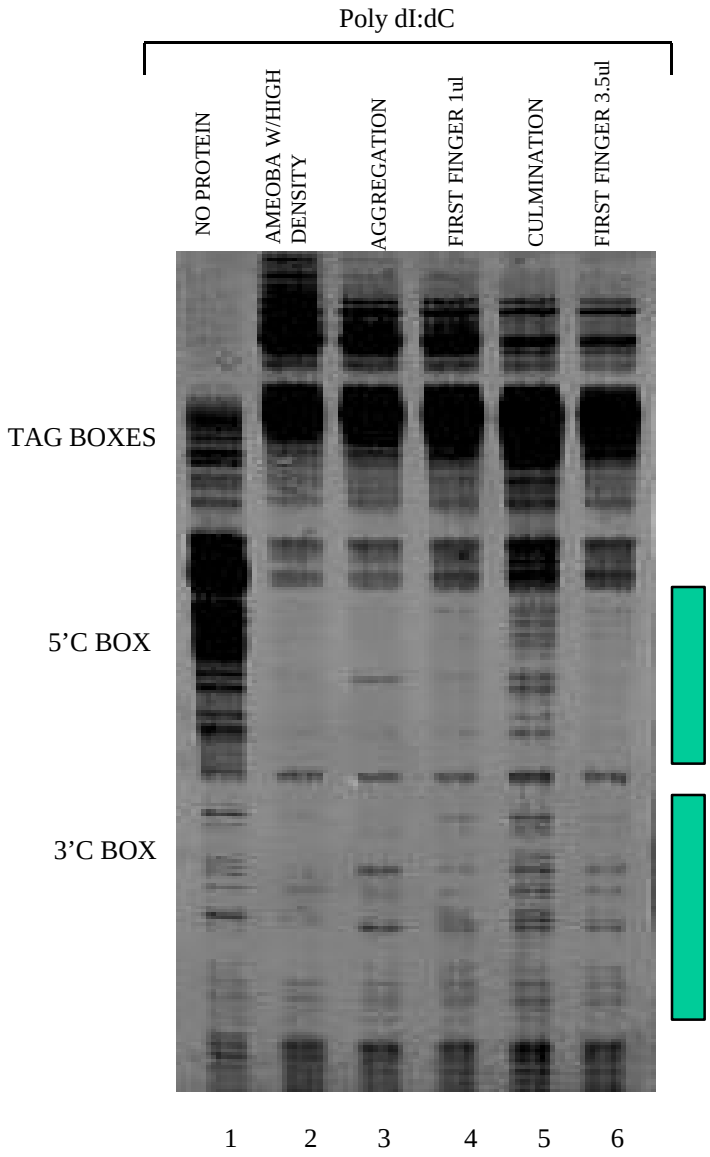


**Figure 3.12.** DNase I footprint assay to detect developmentally regulated footprints. Extracts from ameoba grown at high density, aggregation(8h), first finger(16h) and culmination(21-22h) extracts using the primer extension footprinting technique. p2124 was used as template, 9001100 was applied as the primer and poly dI:dC was used as nonspecific competitor. DNase I digestion time was 8 minutes for lane 1 and 6 min for other lanes. The C and TAG boxes are shown. Footprints are indicated by bars.

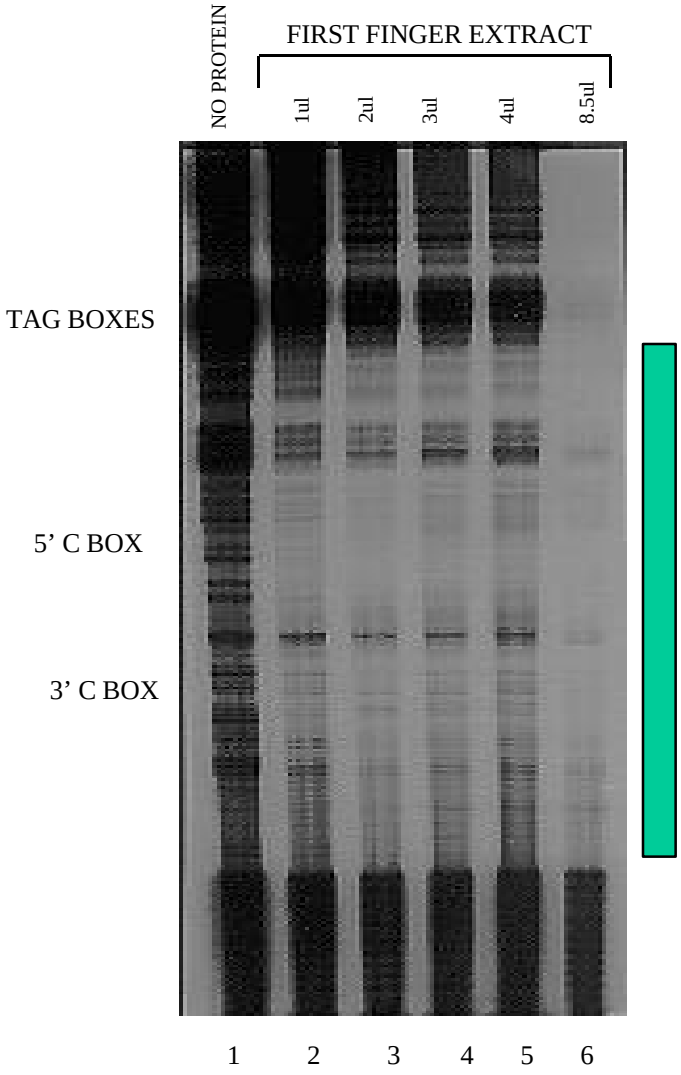


**Figure 3.13.** DNase I footprint assay to test the effect of increasing amounts of first finger(16h) nuclear extract(shown on top). The primer extension footprinting technique was used. p2124 was used as template, 9001100 was applied as the primer and poly dI:dC was used as nonspecific competitor. DNase I digestion time was 8 minutes for lane 1; digestion was 6 min for all other lanes. The C and TAG boxes are shown. Footprints are indicated by a bar.

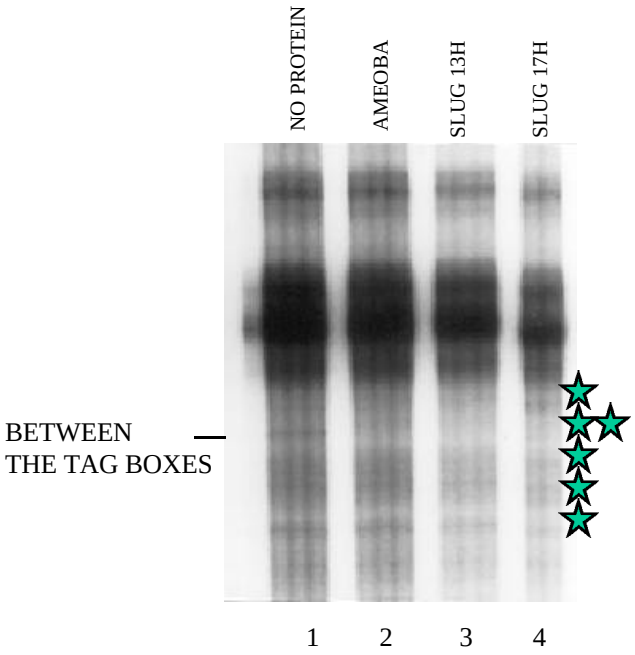


**Figure 3.14.** DNase I footprint assay to detect developmentally regulated regions of protection surrounding the TAG boxes. Nuclear extracts from ameoba, slug (13h) and slug(17h) were tested using the endlabeling footprinting technique. N-9001100K was used as template, poly dI:dC was used as nonspecific competitor. DNase I digestion time was 5 minutes for each lane. The TAG boxes are shown. Footprints are indicated by stars.

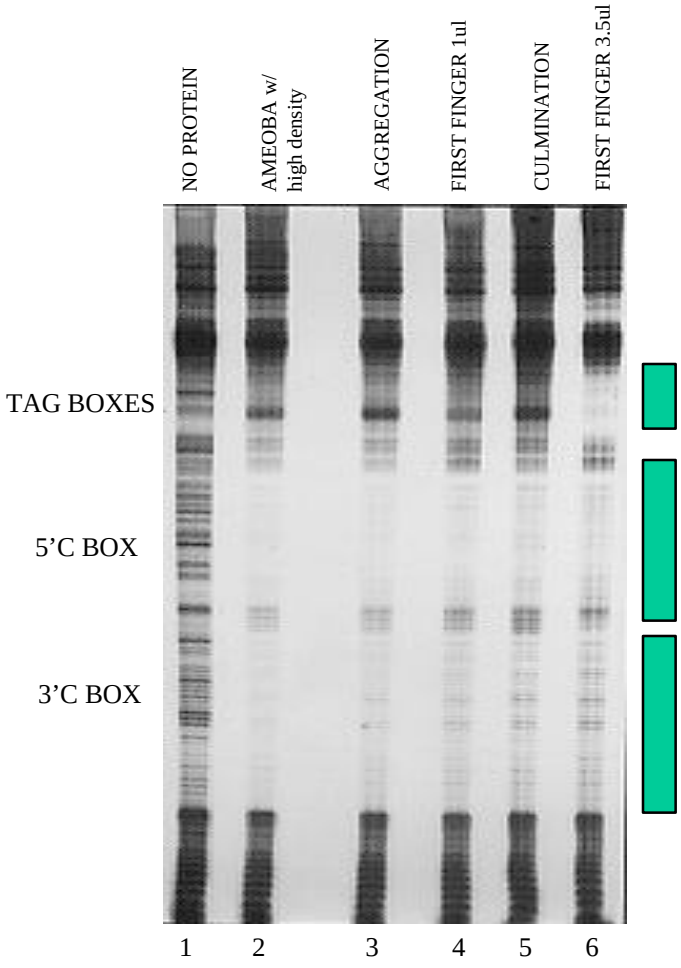


**Figure 3.15.** DNase I footprint assay to detect regions of protection over a large portion of the promoter. Nuclear extracts from ameoba grown at high density, aggregation(8h), first finger(16h) and culmination(21-22h) were tested using the primer extension footprinting footprinting technique. p2124 was used as template, 9001100 was applied as the primer and poly dA:dT was used as nonspecific competitor. DNase I digestion time was 8 minutes for lane 1, and 6 min for other lanes. The C and TAG boxes are shown. Footprints are indicated by bars. Volume of first finger extract used is indicated near the lane label.

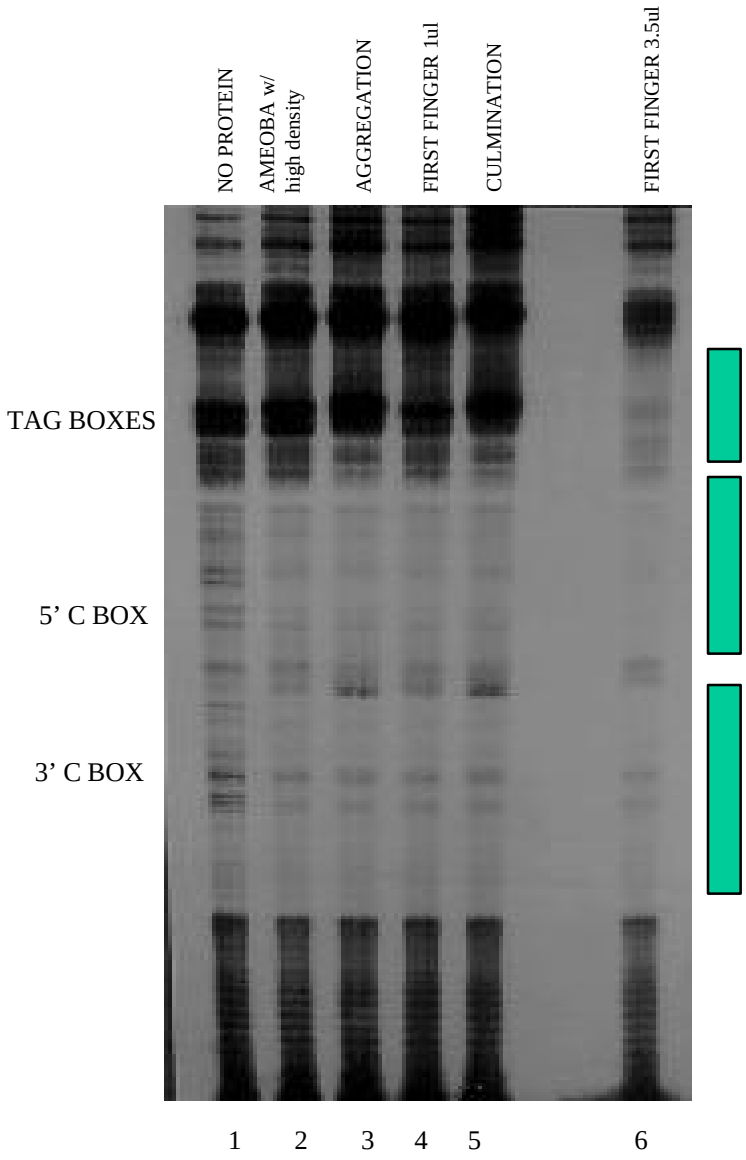


**Figure 3.16.** DNase I footprint assay of ameoba with high density, aggregation(8h), first finger(16h) and culmination(21-22h) extracts using the primer extension footprinting technique. p2124 was used as template, 9001100 was applied as the primer and poly dA:dT was used as nonspecific competitor. DNase I digestion time was 8 minutes for lane 1 and 6 min for other lanes. The C and TAG boxes are shown.. Footprints are indicated by bars. Note that the pattern of protection generated over TAG boxes differs from that seen in Figure 3.15.