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Rapid preparation of single stranded DNA from PCR products by streptavidin induced electrophoretic mobility shift

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ABSTRACT

Streptavidin induced electrophoretic mobility shift was used to prepare single stranded (ss) DNA amplified with the polymerase chain reaction in the presence of a biotinylated and a non-biotinylated primer. A variety of denaturing conditions, including incubation at 95°C in 50% formamide can be used without disrupting the streptavidin–biotinylated-ssDNA complex. Following electrophoresis, pure non-biotinylated DNA can be efficiently recovered from 7 M urea gels because it is well separated from the severely retarded streptavidin–biotinylated-ssDNA complex. Quantitative complexing of biotinylated ssDNA can occur at a streptavidin to DNA molar ratio of 1 or more.

The purification of single stranded (ss) DNA is an important, if not an essential component of many procedures in molecular biology such as strand specific hybridization probing (1), sequencing (2), and Systematic Evolution of Ligands by EXponential Enrichment (SELEX) (3).

Several methods have been described for the generation of ssDNA from double stranded (ds) PCR products. These methods include asymmetric PCR (4), PCR with chemically modified primers that causes the synthesis of strands of unequal length (5), preferential exonuclease digestion of one appropriately modified strand (6,7), and affinity purification (8–10). Only the latter three methods yield ssDNA relatively free of dsDNA PCR products.

For affinity purification, streptavidin immobilized on agarose (9) or magnetic (10) beads was used and the DNA strands were separated by alkaline or heat denaturation. This procedure yields reasonable results but monitoring the purification efficiency requires additional analysis. Furthermore, if one needs to isolate ssDNA of uniform size, additional purification on a high resolution denaturing gel is necessary.

I present here an efficient and rapid method that allows the purification of ssDNA of uniform size from PCR products. This method takes advantage of the remarkable stability of the biotin–streptavidin interaction under strongly denaturing conditions. It is based on the streptavidin induced electrophoretic mobility shift of biotinylated ssDNA on 7M urea-polyacrylamide gels, and combines in one step the streptavidin partition and band purification of ssDNA from a high resolution denaturing gel while the quantity and quality of the ssDNA can be directly observed before purification.

For purification, the DNA is PCR amplified using a biotinylated primer, complementary to the desired single stranded DNA, together with an unmodified primer. PCR product is precipitated with isopropanol at room temperature to remove the bulk of unincorporated primers and reduce the volume, and then is incubated with streptavidin at room temperature for 30 min. The binding mix is then denatured and electrophoresed on an 8% acrylamide, 7 M urea gel. The free ssDNA (non-biotinylated lower band) is then purified by passive elution from the crushed acrylamide gel.

PCR reactions were set in 100 µl with 10–100 nM of ssDNA template (5′-AGATGCCTGTCGAGCATGCTG[N]$_{40}$GTAGCTAAACAGCTTTGTCGACGGG, where N is a position that each base occurs with a frequency of 0.25); 6.4 µM each of 3′ (5′-CCCGTCGACAAAGCTGTTTAGCTAC) and 5′ (5′-CCGAAGCTTAATACGACTCACTATAGGGAGATGCCTGTCGAGCATGC-TG or 5′-ZZZAGATGCCTGTCGAGCATGCTG where Z = biotin) primers; 1 mM each deoxynucleoside triphosphate; 20 µCi [α-^{32}P]dCTP (800 Ci/mmol, NEN); and 2.5 U *Taq* DNA polymerase (Perkin Elmer). PCR conditions were an initial denaturation at 93°C for 3.5 min followed by 15 amplification cycles (30 s denaturation at 93°C; 30 s annealing at 53°C; and 1 min elongation at 72°C). At the end of the amplification the dsDNA PCR product was phenol extracted and isopropanol precipitated at room temperature (2). The precipitated DNA was resuspended in 10 mM Tris–HCl pH 7.5, 50 mM NaCl, 1 mM EDTA, and 2–5 mg/ml (27–67 µM) streptavidin (Pierce, affinity-purified, Cat. No. 21122) to achieve 1:2–1:10 molar ratio of biotinylated primer to streptavidin. The streptavidin–DNA mixture was incubated at room temperature for 30 min and then it was treated and electrophoresed as in Figure 1.

Following electrophoresis on denaturing gels (Fig. 1), biotinylated-DNA–streptavidin complexes were clearly seen and were well separated from free DNA. As expected, the complex formation required the presence of both biotin and streptavidin (Fig. 1A, lanes 6, 8, 10, 12 and 14). The unshifted ssDNA band was routinely excised and the DNA was recovered from the crushed gel by glass-wool filtration and ethanol precipitation.

In Figure 1, two streptavidin–biotinylated-DNA complexes are seen. The top complex probably contains two biotinylated-ssDNA molecules per streptavidin molecule, while the lower complex contains only a single biotinylated-ssDNA molecule per streptavidin molecule. This hypothesis is consistent with the observation that at lower streptavidin concentrations (or high DNA to streptavidin ratios) the upper complex is abundant while at high streptavidin concentrations (or low DNA to streptavidin ratios) the lower

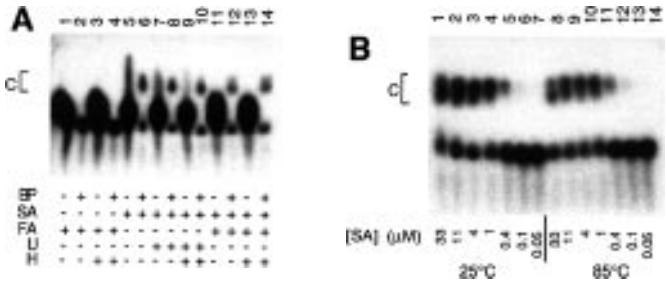

Figure 1. Electrophoretic separation of the streptavidin–ssDNA complexes from the non-biotinylated ssDNA. (**A**) Survey of conditions for treating the streptavidin–DNA mixtures prior to electrophoresis. The conditions tested are indicated under each lane. For each lane, 2 µl of precipitated PCR product (5.12 pmol/µl; 6650 c.p.m./pmol) were mixed with streptavidin (2 mg/ml in 10 mM Tris–HCl pH 7.5, 50 mM NaCl, 1 mM EDTA) at a molar ratio 10:1 (streptavidin:DNA). Following 30 min incubation at room temperature, the incubation mixtures were supplemented with an equal volume of 2× formamide dye (lanes 1–4 and 11–14), or an equal volume of 7 M urea and 1/6 final volume of 6× ficoll loading dye (lanes 7–10), or with 1/6 volume of 6× ficoll dye (lanes 5 and 6). The samples were then loaded onto an 8% acrylamide, 7 M urea gel without heating (lanes 1, 2, 5–8, 11 and 12), after 1.5 min incubation at 85°C (lanes 3, 4, 13 and 14), or after 1.5 min incubation at 95°C (lanes 9 and 10), and electrophoresed at room temperature at 15 W constant power. After electrophoresis the gel was ethidium bromide stained, photographed and exposed to Kodak X-OMAT AR film for 5.5 h at –70°C. Only the autoradiogram is shown. (**B**) Effect of streptavidin concentration on the electrophoretic separation of streptavidin–ssDNA complexes from non-biotinylated ssDNA. As in (A), 2 µl of precipitated PCR product was mixed with streptavidin. The final concentration (in µM) of streptavidin is indicated under each lane. Following 30 min incubation at room temperature, the incubation mixtures were supplemented with an equal volume of 2× formamide dye and loaded onto an 8% acrylamide, 7 M urea gel without heating (lanes 1–7), or after 1.5 min incubation at 85°C (lanes 8–14). The gel was electrophoresed and processed as above. Symbols C, BP, SA, FA, U and H indicate ssDNA complexes: Biotinylated Primer, Streptavidin, Formamide, Urea and Heat, respectively.

complex is abundant. Since only two streptavidin–biotinylated-DNA complexes are seen, it is possible that two of the four biotin binding sites on streptavidin are inaccessible for binding to the particular biotinylated-ssDNA used under these experimental conditions.

Since only one primer was biotinylated, only half of the denatured DNA partitioned in a complex. The streptavidin–biotinylated-DNA complex was very stable and was not dissociated by electrophoresis in 7 M urea, by incubation at 85°C for 1.5 min in 50% formamide (Fig. 1A, lane 14), or by incubation at 95°C in 3.5 M urea (Fig. 1A, lane 10). The two DNA strands were readily dissociated upon electrophoresis in 7 M urea and prior denaturation was not necessary (Fig. 1A, lanes 5 and 6).

Figure 1B shows the formation of the streptavidin–biotinylated-DNA complex as a function of streptavidin concentration. The results were the same either with or without heating for 1.5 min at 85°C prior to electrophoresis.

As shown in Figure 2, complex formation increased with increasing streptavidin concentration and plateaued at ~45% of total input DNA. The plateau starts at ~10^{-6} M streptavidin, which corresponds to a streptavidin to DNA molar ratio of 0.6. The minimum theoretical streptavidin to DNA molar ratio is 0.25, since there are four biotin binding sites per streptavidin molecule. Consistent with the complexes seen, possibly two of the four biotin binding sites are sterically inaccessible under these experimental conditions.

At the plateau, the remaining free DNA (presumably the non-biotinylated single-strand) constitutes ~30% of the total. One

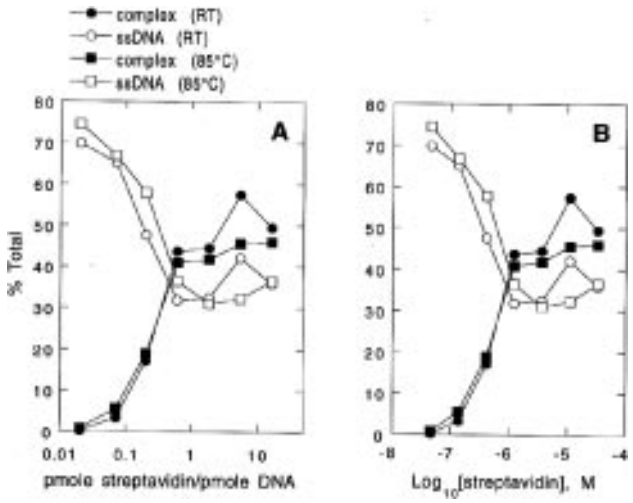

Figure 2. Streptavidin titration curves. Gel electrophoresis was set as in Figure 1B. The complexed and free ssDNA was then quantitated by densitometry. The results are expressed as per cent of total density, where total is the sum of densities of the complexed and the free ssDNA. The same density values were graphed as a function of the molar ratio of streptavidin to DNA (**A**) or as a function of streptavidin concentration (**B**). The symbols used for the free and complexed ssDNA at each temperature are shown.

explanation for the lower than expected amount of free ssDNA is that shorter PCR products form a smear below the free ssDNA band, while their biotin-labeled complements still form streptavidin complexes that migrate with the streptavidin complexes containing full length DNAs. Consistent with this explanation, overexposed autoradiograms contain smears below the full length ssDNA.

In conclusion, the partitioning of biotinylated-DNA and the band purification of non-biotinylated ssDNA can be combined in a single step resulting in higher yield with less effort. This can be achieved by PCR amplification of DNA, using any template/primer set, in the presence of a biotinylated primer, isopropanol precipitation of the DNA, addition of streptavidin at a molar ratio of at least 2:1 (streptavidin:DNA), followed by gel electrophoresis on a denaturing gel and recovery of the ssDNA by band excision and elution from the crushed gel.

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