Suggested Protocols For Purification of Genomic DNA For Southern Blots and PCR Using Prep-A-Gene® DNA Miniprep Kit

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This is a recommended protocol for the purification of genomic DNA for Southern blots and PCR using the Prep-A-Gene DNA purification kit. Read the Prep-A-Gene DNA purification systems instruction manual for recommended use of the Prep-A-Gene matrix and buffers.

Great care must be taken when purifying genomic DNA. Use wide bore pipette tips and mix all solutions by swirling or gentle inversion to minimize shearing of the genomic DNA.

1. Ten to twelve micrograms of undigested genomic DNA were electrophoresed on a 0.6% low melting temperature agarose gel.
2. The gel slice containing the entire sample of genomic DNA is placed in a microcentrifuge tube and water is added to the gel slice so that upon melting, the agarose concentra tion is equal to or less than 0.5% (Example, Gel slice weighed 138 mg — assume weight is equivalent to 138 µl volume. Add 27.6 µl of deionized H_2O to bring the total volume to 165.6 µl and a final agarose concentration of 0.5%.)
3. Melt the agarose at 65 ˚C for 10 minutes. After the agarose has melted, equilibrate the temperature of the solution to 37 ˚C for 1-2 minutes.
4. Prep-A-Gene DNA purification kit binding buffer (sodium perchlorate) is added at 3x volume to the melted agarose. Incubate at 45 ˚C for 2-5 minutes.

Manufacturer's Note: Alternatively, depending on the application, a 3x volume of binding buffer could have been added directly to the undigested genomic DNA solution before step 1 of this procedure, or non-low melting temperature agarose could be used. A 3x volume of binding buffer will dissolve the agarose at 37-55 ˚C.

5. Add Prep-A-Gene matrix at 5 µl matrix/µg DNA. As the matrix begins to bind DNA, small pellets (flocculent precipitate) begin to form. Incubate at room temperature for 10 minutes. Mix periodically by gentle inversion.
6. Centrifuge for 30 seconds at high speed to pellet the matrix and discard the supernatant.
7. Add 25 X pellet volume of Prep-A-Gene DNA purification binding buffer (sodium perchlorate) and resuspend the pellet by breaking up the pellet with a pipette tip and gentle inversion.
8. Centrifuge for 30 seconds at high speed to pellet the matrix and discard the supernatant.
9. Repeat steps 7 and 8.
10. Add 25 X pellet volume of Prep-A-Gene wash buffer and resuspend the pellet by breaking up the pellet with a pipette tip and gentle inversion.
11. Centrifuge for 30 seconds at high speed to pellet the matrix and discard the supernatant.
12. Repeat steps 10 and 11 twice.
13. Make sure all the wash buffer is removed. (It may be nec essary to spin the tubes again, and air dry or lyophilize the pellet.)
14. Resuspend the pellet by adding one pellet volume of Prep-A-Gene elution buffer. Incubate the suspension for 5 minutes at 45 ˚C.
15. Centrifuge for 50 seconds to pellet the matrix. Save the supernatant to another tube. Step 14 can be repeated to increase the yield of the DNA from the matrix.
16. Analyze a portion of the purified genomic DNA on an agarose gel by ethidium bromide staining.

We have routinely purified genomic DNA using the Prep-A-Gene DNA purification kit that is free of RNA and demonstrates minimal shearing of the DNA.

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