

ISOLATION OF DNA FRAGMENTS FROM POLYACRYLAMIDE GELS  
*"crush and soak" method*

1. Run and stain the polyacrylamide gel as usual.
2. Use a long-wave UV lamp to locate the DNA band of interest.
3. Cut out the band, using a sharp razor blade.
4. Place the gel slice on a glass plate and chop it into fine pieces with a razor blade. Transfer the pieces to a small test tube and add 1 volume of elution buffer (0.5 M ammonium acetate and 1 mM EDTA [pH8.0]).
5. Cap the tube and incubate at 37°C overnight, if possible on a rotating wheel.
6. Centrifuge the sample at 10,000 g for 10 minutes at 20°C. Recover the supernatant. Be careful to avoid transferring fragments of acrylamide (a drawn-out pasteur pipette works well).
7. Add an additional 0.5 volume of elution buffer to the pellet, vortex briefly, and recentrifuge. Combine the two supernatants.
8. Remove any remaining fragments of acrylamide by passing the supernatant through a small column of glass wool contained in the tip of a pasteur pipette.
9. Precipitate the DNA with ethanol.
10. Redissolve the DNA in 200  $\mu$ l of TE. Add 25  $\mu$ l of 3 M sodium acetate (pH 5.2) and reprecipitate the DNA.
11. Rinse the pellet once with 70% ethanol; dry briefly under vacuum and resuspend in a small volume of TE (pH 7.9).

**Reference:** *Maxam, A.M. and W. Gilbert (1977) DNAs 74:560.*