

DNA Isolation for Low-Melting Point Agarose (using elu-tip method)

1. Excise fragment from gel and estimate volume.
2. Add 1/100 volume of 1 M Tris pH 7.5, 1/50 volume of 0.5 M EDTA, and 1/100 volume of 5M NaCl.
3. Incubate at 68°C for 10 minutes.
4. Vortex and remove to a small tube.
5. Incubate at 37°C for 5 minutes.
6. Phenol extract 2-3 times (phenol at 42°C, spin at 13 K for 2 minutes.)
7. Ether extract 1 time. Place tubes at 65°C, then speed-vac 2 to 5 minutes. Repeat procedure about 4 times to get rid of residual ether.
8. Ethanol precipitate DNA and suspend pellet in 1 ml of low salt buffer (from elu-tip column protocol)
9. Following the elu-tip protocol booklet, wash the column by pushing 5 ml of *low* salt buffer through the matrix at a rate of 0.5-1.0 ml/minute. The column may be incubated in the *low* salt buffer ³ 2 hours to improve recovery.
10. Load DNA sample onto the column slowly (1-2 drops/second). NOTE: When recovering DNA from low-melt temperature agarose, use of the pre-filter is not recommended. Consult the protocols booklet for specific parameters of different types of nucleic acid purification (i.e. DNA purification when LMP agarose isn't used).
11. Wash the column with 2-3 mls of pre-warmed (42°C) *low* salt buffer.
12. Elude DNA with 0.4 ml of *high* salt buffer. Do a total of 2-3 washes as desired to be certain of good recovery.
13. Ethanol precipitate DNA and resuspend in TE or dH₂O.

SOLUTIONS

For Recovery of DNA from LMP agarose:

Low Salt Buffer: per 100 ml

0.2 M NaCl 4 ml of 5M NaCl

20 mM Tris HCL 2 ml of 1M Tris

1.0 mM EDTA 0.02 ml of 0.5M

pH 7.4

High Salt Buffer: per 100 ml

1.0 M NaCl 20 ml of 5M NaCl

20 mM Tris HCL 2 ml of 1M Tris

1.0 mM EDTA 0.02M of 0.5M

pH 7.4