Purification of nucleic acids from agarose gels

Recovery of DNA fragments from agarose gels is necessary when undesired side-products cannot be removed or inactivated ahead of sensitive downstream applications such as cloning or sequencing.
The DNA can be electro-eluted into a trough excised ahead of the band (McDonell et al., 1977), or more conveniently, the band can be excised directly.
To remove the agarose, fast flush extraction (Grey and Brendel, 1992) (*Alternative A*) or chaotropic salts such as guanidinium thiocyanate (Matitashvili and Zavizion, 1997) (*Alternative B*) are commonly used. The latter, as a silica-based method, being convenient, at the expense of lower recovery and higher cost. Fast flush extraction is thus preferred for large sample sets.

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This is a bench card. Full protocol available online.

Procedures

›› ***Separation and excision of the desired DNA fragment from agarose gels***

(1.) Resolve the desired DNA fragment on a 0.6–1.2% agarose gel in TAE or lithium acetate buffer. Load at least 200 ng per lane.

> ***Critical:*** To avoid carry-over of DNA, use fresh running buffer and leave empty lanes between samples.

> ***Critical:*** Avoid TBE buffer if possible, since borate inhibits downstream enzymatic reactions.

(2.) Visualize the gel using a blue light or long-wave UV source in a darkroom or gel box.

> ***Critical:*** Always use long-wave UV (> 400 nm) and minimize exposure. Avoid pre-imaging gels under UV to prevent DNA damage and pyrimidine dimer formation.

(3.) Excise the desired band with a clean surgical blade. Trim excess agarose to minimize the slice size.

(4.) Transfer the gel slice to a clean microcentrifuge tube.

A › ***Fast flush extraction***

(1.) Place the gel slice into a spin column. Centrifuge at 15 000 × *g* for 1 min. Collect the flow-through.

(2.) Rotate the column 180° and spin again at 15 000 × *g* for 1 min.

> ***Quality assurance:*** Use a blue light flashlight to check for residual fluorescence. If DNA remains, add 50 µL 5 mM Tris pH 8.0 and repeat the spin. Expect a faint greenish glow in the eluate.

(3.) Combine all flow-throughs. Discard the column.

(4.) *Optional:* Further purify by silica spin column or ethanol precipitation SOP 0021.

B › ***Extraction with guanidinium thiocyanate***

- ☐ R0135 Buffer QG (Extraction buffer), 0.5 mL
- ☐ Isopropyl alcohol

(1.) Weigh the gel slice.

(2.) Add 350 µL Buffer QG per 100 mg gel. If the gel slice weighs less, supplement with electrophoresis buffer to 100 mg.

(3.) Melt the agarose at 40–60 °C. Mix occasionally. Cool to room temperature.

(4.) If the phenol red indicator turns red, add 10 µL 3 M sodium acetate pH 5.0.

> ***Critical:*** Do not skip this step—binding will fail at neutral or basic pH.

(5.) Add 150 µL isopropanol per 100 mg agarose.

(6.) Bind DNA to silica SOP 0021. Skip Buffer PB.

List of references

M. Grey and M. Brendel, *Curr. Genet* **22**(1), 83—84 (1992).

E. Matitashvili and B. Zavizion, *Anal. Biochem.* **246**(2), 260—262 (1997).

M. McDonell, M. Simon, and F. Studier, *J. Mol. Biol.* **110**(1), 119—146 (1977).

Recipe (available online) Troubleshooting (available online) Notes (available online)

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