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.

Risk assessment

– Transilluminators emit ultraviolet radiation, which can damage unprotected eyes and skin.
– Guanidine salts form TOXIC GASES with bleach or strong acid
▷ Wear gloves, safety glasses, lab coat
□ DO NOT add bleach or acidic solutions directly into sample waste

Reviewed: Nov 26, 2023

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.

> ***Hint:*** Clean the blade between samples by stabbing into an unused gel area near the top.

(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.

> ***Note:*** Most of the liquid will be extracted from the gel into the collection tube.

> ***Hint:*** Alternatively, puncture the bottom of a 0.5 mL tube with a needle, add a small cotton plug, and place it inside a 1.5 mL tube. Spin at 5 000 × *g* for 5 min.

(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 0021.

> ***Note:*** The eluate can be used directly for ligation, PCR, sequencing, or probe labeling.

[AMI+17]

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

☐ Buffer QG (Extraction buffer), 0.5 mL ⟨*R*⟩	☐ 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 0021. Skip Buffer PB.

> ***Hint:*** If volume exceeds 700 µL, reload after first spin. Silica membranes bind up to 25 µg DNA.

Analyses

○ Measure nucleic acid concentration and purity at 260 nm, 280 nm, and 230 nm.

Nucleic acid	A260 = 1.0	A260/A280	A260/A230
dsDNA	50 ng/µL	1.8–1.9	2.0–2.2

> ***Note:*** Extinction coefficients and purity ratios depend on sequence. A260/A280 varies 0.2–0.3 with pH.

> ***Quality assurance:*** High A230 absorbance indicates residual guanidine contamination.

Troubleshooting

Separation and excision of the desired DNA fragment from agarose gels

In Step 1:

– Fragment resolves into multiple bands after digestion.
  ◦ Heat-inactivate the restriction enzyme or purify before gel loading. Some enzymes bind to DNA and alter its migration.

Extraction with guanidinium thiocyanate

In Step 5:

– Mixture appears cloudy or gelatinous
  ◦ The gel may be incompletely dissolved. Proceed with column binding, then flush with 0.5 mL Buffer QG. Let stand 1 min before spinning.

Recipes

Buffer QG (Extraction buffer), pH 6.6

Amount	Ingredient		Stock	Final
100 g	Guanidine thiocyanate [593-84-0]		118.16 g/mol	5.5 M
3 mL	Tris-Cl, pH 7.4	R0055	1 M	20 mM
45 µL	Phenol red	[143-74-8]	10 g/L	30 mg/L
To 154 mL	Water, reagent-grade			

Prepare Buffer QG in the bottle without weighing. Add 117 mL water per 100 g guanidinium thiocyanate, bring to 65 °C to dissolve. Adjust pH and final volume. Dispense into 15 mL aliquots.

Buffer QG (Extraction buffer)

5.5 M Guanidine thiocyanate, 20 mM Tris-Cl, 30 mg/L Phenol red, pH 6.6

DANGER

Serious eye damage

Date: Sign: R0135

List of references

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Change log

2021-04-01	Nick Coleman	Original protocol.
2023-11-26	Benjamin C. Buchmuller	Adaptation as SOP.