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 from the excised slice in a dialysis bag (McDonell et al., 1977), or into a trough cut ahead of the band (Green and Sambrook, 2012), 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: Oct 27, 2026

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 (365 nm) or a blue-light source 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 SOP 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), pH 6.6 (300 µL / 1 rxn) ◇ R0135
  - • *or*: Buffer QG, QIAGEN
- ☐ 3 M NaOAc, pH 5.2 (10 µL / 1 rxn, *optional*) ◇ R0045
- ☐ Isopropyl alcohol
- ☐ Spin column, silica, about 10 µg dsDNA capacity, < 50 µL elution (1 / 1 rxn)

(1.) Weigh the gel slice.

(2.) Add 300 µL Buffer QG per 100 mg gel, or 600 µL for gels above 2%. If the gel slice weighs less, supplement with electrophoresis buffer to 100 mg.

> ***Critical:*** Process at most 400 mg of gel per column. Split larger slices over several columns.

(3.) Melt the agarose at 40–50 °C for 10 min, or until the slice has dissolved completely. Vortex every 2–3 min. Cool to room temperature.

> ***Hint:*** Gels above 2% need longer. Dissolve the agarose completely before loading the column.

(4.) Check that the mixture is yellow. If it is orange or violet, add 10 µL 3 M sodium acetate pH 5.2 and mix until it turns yellow.

> ***Critical:*** Do not skip this step—binding is efficient only at pH 7.5 or below, and the indicator turns orange or violet above it.

(5.) For fragments below 500 bp or above 4 kbp, add 100 µL isopropanol per 100 mg agarose and mix. Between these sizes, isopropanol does not improve the yield.

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

> ***Hint:*** If volume exceeds 700 µL, reload after first spin. The membrane binds about 10 µg DNA.

Analyses

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

Nucleic acid	A_{260} = 1.0	A_{260}/A_{280}	A_{260}/A_{230}
dsDNA	50 ng/µL	1.8–1.9	2.0–2.2

> ***Note:*** Extinction coefficients and purity ratios depend on sequence. A_{260}/A_{280} varies by 0.2–0.3 with pH.

> ***Quality assurance:*** High A_{230} 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.

Materials

Extraction with guanidinium thiocyanate

- ☐ **Per 100 mg of gel: extraction buffer** (300 µL / 1 rxn)
  - **Buffer QG (Extraction buffer)**, pH 6.6 ◇ R0135
  - *or* **Buffer QG**
    QIAGEN, QIAquick Gel Extraction Kit, 28704
- ☐ **Sodium acetate (NaOAc)**, 3 M, pH 5.2 ◇ R0045 (10 µL / 1 rxn, *optional*)
- ☐ **Isopropyl alcohol**
  CAS 67-63-0
- ☐ **Spin column** (1 / 1 rxn), silica, about 10 µg dsDNA capacity, < 50 µL elution
  - **QIAquick spin column**
    QIAGEN, QIAquick Gel Extraction Kit, 28704

Recipes

Buffer QG (Extraction buffer), pH 6.6

Amount	Ingredient		Stock	Final
100 g	Guanidine thiocyanate Skin corrosion (H314)	[593-84-0]	118.16 g/mol	5.5 M
3 mL	Tris hydrochloride (Tris-Cl), pH 7.5	◇ R0055	1 M	20 mM
450 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

Skin corrosion; Acute dermal toxicity; Acute inhalation toxicity; Acute oral toxicity; Aquatic toxicity (long-term)

- ☐ Collect as CYANIDE WASTE
- ☐ Keep alkaline, DO NOT acidify
- ☐ DO NOT wash into sewer

Date: Sign: R0135

List of references

O.-S.J. Abraham, T.-S. Miguel, H.-C. Inocencio, and C.-C. Blondy, *3 Biotech* **7**(3), 180 (2017).

M.R. Green and J. Sambrook, *Molecular cloning : a laboratory manual*, 4th ed. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y, 2012).

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).

Change log

2026-05-21 **Nick Coleman, Benjamin C. Buchmuller** Version 1, 2021 to 2026. The Nick Coleman lab protocol "Purification of DNA in gel slice", adapted as an SOP in 2023.

2026-10-27 **Benjamin C. Buchmuller** Version 2. Credited QIAGEN for Buffer QG and the gel-extraction procedure and Molecular Cloning for electro-elution into a trough, which the McDonell paper does not describe; corrected the Buffer QG volume to 3 gel volumes, the isopropanol volume to 1 gel volume and made it conditional on fragment size, the melting and pH-indicator conditions, the pH limit for binding, the column capacity (10 μg, stated twice and inconsistently before), and the long-wave UV wavelength.

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