DNA isolation from acrylamide gel.v1

Procedures
1. **Stain acrylamide gel** (4-10%, 1.5 mm thick) by immersing it in ethidium bromide for 20-30 minutes.
2. Visualize DNA using the preparative wavelength on the UV box, cut out a piece of acrylamide (as little as possible without losing the DNA) containing the DNA band of interest.
3. Place on parafilm and **rinse gel** with 0.5 ml of 1X TE, drain off all liquid, cut gel into ~1 mm pieces, put into a 1.5-ml eppendorf tube.
4. Add 400 µl Elution Buffer, make sure all fragments submerged, incubate in 37°C shaking incubator overnight.
5. Spin for 5 min, transfer the supernatant (keep out gel fragments) into a fresh 1.5-ml eppendorf tube. Add 16 µl 5 M NaCl and 1 ml 100% ethanol. Vortex, incubated on dry ice for 10 min and spin for 20 min.
6. **Wash the pellet** with 70% ethanol, drain well and vacuum dry. Resuspend the DNA in 15-20 µl of TEE.

Reagents preparation

**Elution buffer**

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>5 M NH₄OAc</td>
<td>1.0 ml</td>
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<tr>
<td>0.5 M EDTA</td>
<td>0.2 ml</td>
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Notes
1. This procedure works well for fragments below 700 bp (down to 24 bp on 10% gel has worked well) but not in the kb range.
2. Check recovery and approximate concentration of isolated DNA on a gel.
3. Gel slice contains ethidium bromide, so do not cut out gel slice and store for isolation late otherwise you risk having nicks in the DNA.