DNA Gel extraction

Under the UV light, cut out the piece of gel you are interested in.

Put in a tube, that is big enough to contain the gel + buffer volume.

Weight the piece of gel and add 100 µl 0.4 M NaCl for 100 mg gel.

Heat the mix at 70°C for 10 to 15 min, or at least until the gel dissolved well.
Check the volume and add the same volume of phenol only pre-heated at 42°C in a waterbath (equilibrated phenol, pH8.0, ultrapure MB grade).

Vortex well at least 30 sec.
Centrifuge, 14 000 rpm, 5 min at RT.

Take the upper layer as quick as possible as the agarose will diffuse rapidly back to the aqueous layer after the centrifuge stopped.

Add 1 vol. of phenol/chloroform pH 8.0
Vortex 10 sec.
Centrifuge, 14 000 rpm, 5 min, RT

Take the upper layer.
Add 1 vol of chloroform
Vortex 10 sec.
Centrifuge, 14 000 rpm, 5 min, RT

Take the upper layer
Add 1 µl Glycoblue
Add 3 vol. 100% EtOH
Incubate 30 min to 2 hours at -80°C

Centrifuge, 14 000 rpm, 20 min, 4°C
Wash the pellet with 70% EtOH
Centrifuge, 14 000 rpm, 5 min, 4°C
Air dry the pellet

Resuspend in H2O PCR Grade.

Add 1/10 vol of 5M NaCl – you can increase the volume (vol) here by adding 0.5 M NaCl so that you loose less sample afterwards when getting the supernatant.

Add 1 vol Phenol/Chloroform/IaA (25:24:1)*
Vortex, 10 sec
Centriguge, 14000 rpm, 5 min, 4°C

Supernatant volume (put the tube on the side to take out the sup)

Add 1 vol chloroform
Vortex, 10 sec
Centrifure, 14000 rpm, 5 min, 4°C

Supernatant volume
1 µl glycoblue
3 vol 100% EtOH
Incubate, 30 min, -80°C or overnight -20°C

If you have small amount of DNA or RNA, it is better to let the sample 2 hours at -80°C.

Centrifuge, 14000 rpm, 20 min, 4°C
Wash with 70% EtOH
Centrifuge , 14000 rpm, 5 min, 4°C
Air dry pellet
Resuspend in H2O PCR grade (DNA) or DEPC-H2O (RNA)

* to precipitate RNA : Phenol pH 4.3 (Sigma, P4682)
  to precipitate DNA/RNA : Phenol pH 8.0 (Sigma, P4557)

Maiwen Caudron-Herger B066 m.caudron@dkfz-heidelberg.de