Recovery of DNA from Agarose

Note: FYI, as kits are now available that permit DNA to be isolated by centrifugation alone.

Agarose gel electrophoresis: Example involves cutting of plasmid to yield two fragments. Requires low melting point agarose as enzyme will only works on agarose that is still in liquid form at low temperature.

Add and incubate B-Agarase enzyme: Cutting out of Et-Br labeled enzyme from gel. Weigh microfuge tube empty, then add gel chunks (deduce weight of chunk by subtracting weight of tube alone.)
Add buffer to agarose and bring up to 65 ^{o}C to melt (for 10 minutes) and cool to 40 ^{o}C (should still be liquid.)
Add agarase enzyme and let incubate for 1 hour. FYI, beta-agasrase and agarose are harmless to most reactions, but Et-Br needs to be removed.

DNA purification: Chill sample for 10 minutes on ice. Solid agarose will spin down. Add sodium acetate and chill again and remaining agarose will ppt.

- Phenol and chloroform can be used to remove agarase if necessary.
- Cold isopropanol and salt will ppt DNA (Et-Br stays in alcohol.)
- Ligation reaction can then be conducted on isolated fragments and assessed via gel analysis.

Analysis of results: Note that ligation gives many novel products, many of which are larger than the original plasmid that was cut.

Alternate methods

Electro-elution: agarose plug is placed into dialysis bag (with buffer,) and placed into electrophoresis chamber (with buffer.) Collect dialysis contents and put through column. Contents are then Et-OH precipitated.

Phenol extraction: FYI

Centrifugation: FYI CeCl gradient