

Recovery of DNA from agarose gels

- a number of methods is available (only the most commonly used are listed):
 - **electrophoresis on DEAE-cellulose membranes:**
 - membranes positively charged; will thus bind DNA (DEAE= diethylaminoethyl)
 - slit is made next to DNA band, membrane inserted, and voltage applied to let DNA run into the membrane
 - membrane is washed in H₂O and DNA eluted with high-salt buffer (1 M NaCl)
 - advantage: quick and inexpensive
 - disadvantage: DNA has to be concentrated after extraction by alcohol precipitation (which also removes salt that would inhibit enzymatic reactions)
 - use of **low-melting-temperature agarose:**
 - this is a chemically modified agarose that melts and gels at lower temperatures; is still liquid at temperatures above ~ 30 °C
 - DNA can be extracted from melted agarose by phenol-chloroform or
 - melted agarose with the DNA in it can be directly added to enzymatic reactions (ligation, restriction digest etc); however, the efficiency of these reactions will usually be lower as with purified DNA
 - instead of melting the gel, the gel can be degraded with the enzyme agarase before phenol-chloroform extraction; is gentler than melting and used for very large DNAs
 - disadvantages: gels difficult to handle; expensive
 - **column chromatography:**
 - DNA recovered using the methods mentioned above is often contaminated by poorly defined substances that can inhibit enzymatic reactions and limit the use of the DNA in these reactions; these impurities can be removed by column chromatography, e.g. anion-exchange chromatography using DEAE (see above); however, we will use a procedure explained below that recovers and purifies the DNA at the same time
 - **using silica membranes:** DNA binds strongly to silica (SiO₂) and also to glass beads (silica is the main component of glass!) at high salt concentrations; this property is exploited in a purification kit from Qiagen (MinElute Gel Extraction kit) that we will use
 - briefly, the gel slice with the DNA is dissolved in a solution containing a chaotropic salt (a salt that interferes with the formation of hydrogen bonds; will disrupt the interaction of DNA with water, thus reducing its solubility and promoting binding to silica)
 - the solution is then loaded onto a column with a silica membrane that will bind the DNA
 - contaminants and salt are washed away by the use of different wash buffers
 - the DNA is finally eluted with a small volume of H₂O or TE buffer
 - the columns used are "spin columns", meaning that the solution can be rapidly recovered from the columns by brief centrifugation
 - advantages: ready-to-use DNA that does not require further concentration or purification
 - for detailed step-by-step instructions, see the MinElute Handbook that comes with the kit