DNA isolation methods

Many different methods and technologies are available for the isolation of genomic DNA. In general, all methods involve disruption and lysis of the starting material followed by the removal of proteins and other contaminants and finally recovery of the DNA. Removal of proteins is typically achieved by digestion with proteinase K, followed by salting-out, organic extraction, or binding of the DNA to a solid-phase support (either anion-exchange or silica technology). DNA is usually recovered by precipitation using ethanol or isopropanol. The choice of a method depends on many factors: the required quantity and molecular weight of the DNA, the purity required for downstream applications, and the time and expense.

Several of the most commonly used methods are detailed below, although many different methods and variations on these methods exist (a comparison of methods is shown in Figure 7, page 13). Home-made methods often work well for researchers who have developed and regularly use them. However, they usually lack standardization and therefore yields and quality are not always reproducible. Reproducibility is also affected when the method is used by different researchers, or with different sample types.

The separation of DNA from cellular components can be divided into four stages:

1. Disruption
2. Lysis
3. Removal of proteins and contaminants
4. Recovery of DNA

In some methods, stages 1 and 2 are combined.

Preparation of crude lysates

An easy technique for isolation of genomic DNA is to incubate cell lysates at high temperatures (e.g., 90°C for 20 minutes), or to perform a proteinase K digestion, and then use the lysates directly in downstream applications. Considered "quick-and-dirty" techniques, these methods are only appropriate for a limited range of applications. The treated lysate usually contains enzyme-inhibiting contaminants, such as salts, and DNA is often not at optimal pH (1). Furthermore, incomplete inactivation of proteinase K can result in false negative results and high failure rates. It is not recommended to store DNA prepared using this method, as the high levels of contamination often result in DNA degradation.

Salting-out methods

Starting with a crude lysate, "salting-out" is another conventional technique where proteins and other contaminants are precipitated from the cell lysate using high concentrations of salt such as potassium acetate or ammonium acetate (2). The precipitates are removed by centrifugation, and the DNA is recovered by alcohol precipitation. Removal of proteins and other contaminants using this method may be inefficient, and RNase treatment, dialysis, and/or repeated alcohol precipitation are often necessary before the DNA can be used in downstream applications. DNA yield and purity are highly variable using this method.

Organic extraction methods

Organic extraction is a conventional technique that uses organic solvents to extract contaminants from cell lysates (3, 4). The cells are lysed using a detergent, and then mixed with phenol, chloroform, and isoamyl alcohol. The correct salt concentration and pH must be used during extraction to ensure that contaminants are separated into the organic phase and that DNA remains in the aqueous phase. DNA is usually recovered from the aqueous phase by alcohol precipitation. This is a time-consuming and cumbersome technique. Furthermore, the procedure uses toxic compounds and may not give reproducible yields (5). DNA isolated using this method may contain residual phenol and/or chloroform, which can inhibit enzyme reactions in downstream applications, and therefore may not be sufficiently pure for sensitive downstream applications such as PCR (6). The process also generates toxic waste that must be disposed of with care and in accordance with hazardous waste guidelines. In addition, this technique is almost impossible to automate, making it unsuitable for high-throughput applications.

Cesium chloride density gradients

Genomic DNA can be purified by centrifugation through a cesium chloride (CsCl) density gradient. Cells are lysed using a detergent, and the lysate is alcohol precipitated. Resuspended DNA is mixed with CsCl and ethidium bromide and centrifuged for several hours. The DNA band is collected from the centrifuge tube, extracted with isopropanol to remove the ethidium bromide, and then precipitated with ethanol to recover the DNA. This method allows the isolation of high-quality DNA, but is time consuming, labor intensive, and expensive (an ultracentrifuge is required), making it inappropriate for routine use. This method uses toxic chemicals and is also impossible to automate.

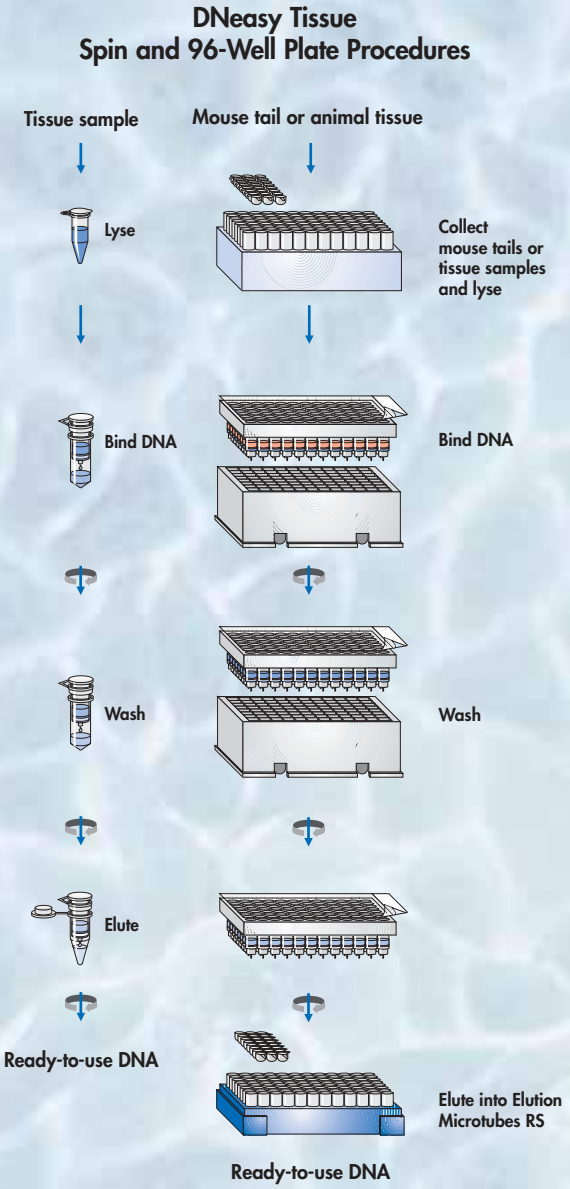

Figure 4. *The DNeasy Tissue spin and 96-well plate procedures.*

Anion-exchange methods

Solid-phase anion-exchange chromatography is based on the interaction between the negatively charged phosphates of the nucleic acid and positively charged surface molecules on the substrate. DNA binds to the substrate under low-salt conditions, impurities such as RNA, cellular proteins, and metabolites are washed away using medium-salt buffers, and high-quality DNA is eluted using a high-salt buffer. The eluted DNA is recovered by alcohol precipitation, and is suitable for all downstream applications.

Anion-exchange technology completely avoids the use of toxic substances, and can be used for different throughput requirements as well as for different scales of purification. The isolated DNA is sized up to 150 kb, with an average length of 50–100 kb. QIAGEN offers QIAGEN Genomic-tips for the purification of high-molecular-weight DNA.

Silica-based methods — DNeasy Tissue Kits

DNeasy Tissue technology provides a simple, reliable, fast, and inexpensive method for isolation of high-quality DNA. This method is based on the selective adsorption of nucleic acids to a silica-gel membrane in the presence of high concentrations of chaotropic salts (Figure 4). Use of optimized buffers in the lysis procedure ensures that only DNA is adsorbed while cellular proteins, and metabolites remain in solution and are subsequently washed away. This is simpler and more effective than other methods where precipitation or extraction is required. Ready-to-use DNA is then eluted from the silica-gel membrane using a low-salt buffer. No alcohol precipitation is required, and resuspension of the DNA, which is often difficult if the DNA has been over-dried, is not required.

DNeasy Tissue Kits are designed for rapid isolation of pure total DNA (genomic, viral, and mitochondrial) from a wide variety of sample sources, including fresh and frozen animal cells and tissues, yeasts, and blood. DNA purified using DNeasy Tissue Kits is free from contamination and enzyme inhibitors and is highly suited for applications such as Southern blotting, PCR, real-time PCR, RAPD, RFLP, and AFLP analyses. DNeasy Tissue Kits are available in convenient spin-column or 96-well formats, suitable for a wide range of throughput needs.

Genomic DNA isolated using DNeasy Tissue technology is up to 50 kb in size, with an average length of 20–30 kb. DNA of this length is particularly suitable for PCR analysis as well as Southern blotting analysis (7–13). Silica-gel spin technology is not suitable if genomic DNA >50 kb is required for certain cloning or blotting applications. QIAGEN recommends the use of QIAGEN Genomic-tips for these applications.

The DNeasy Tissue procedure is suitable for both very small and large sample sizes, from as little as 100 cells up to 5 x 10^6 cells. In order to obtain optimal DNA yield and quality, it is important not to overload the DNeasy System, as this can lead to significantly lower yields than expected (Figure 5). Overloading the DNeasy System can also adversely affect the purity of the DNA (Figure 6).

The DNeasy Tissue procedure is also highly suited for purification of DNA from very small amounts of starting material. If the sample has less than 5 ng DNA (<10,000 copies), 3–5 ng carrier DNA (a homopolymer such as poly dA, poly dT, or gDNA) should be added to the starting material. Ensure that the carrier DNA does not interfere with the downstream application.

Effect of Sample Size on DNA Yield

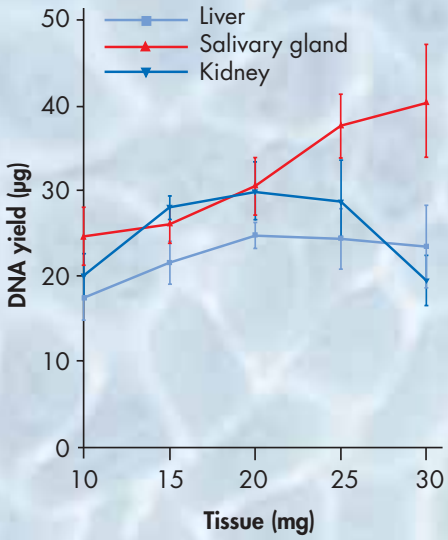

Figure 5. *DNA was purified from tissue samples pooled from 10 mice using the DNeasy Tissue protocol for rodent tails. DNA yield was determined spectrophotometrically. Each point represents the mean and standard deviation from 10 preparations.*

Effect of Sample Size on DNA Purity

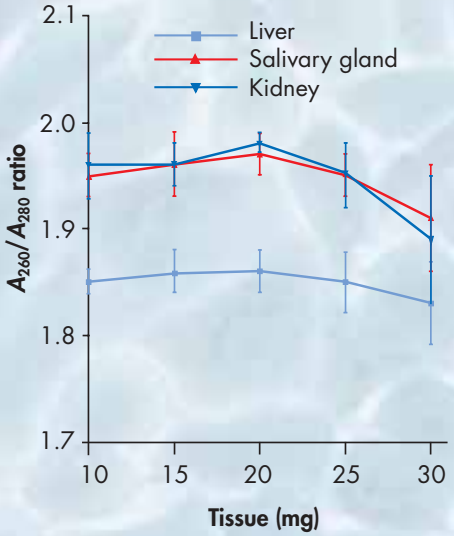

Figure 6. *The DNA purity of the samples described in Figure 5 was determined spectrophotometrically by measuring the A_{260}/A_{280} ratio.*

DNA Purity and Time Required for Different Isolation Methods

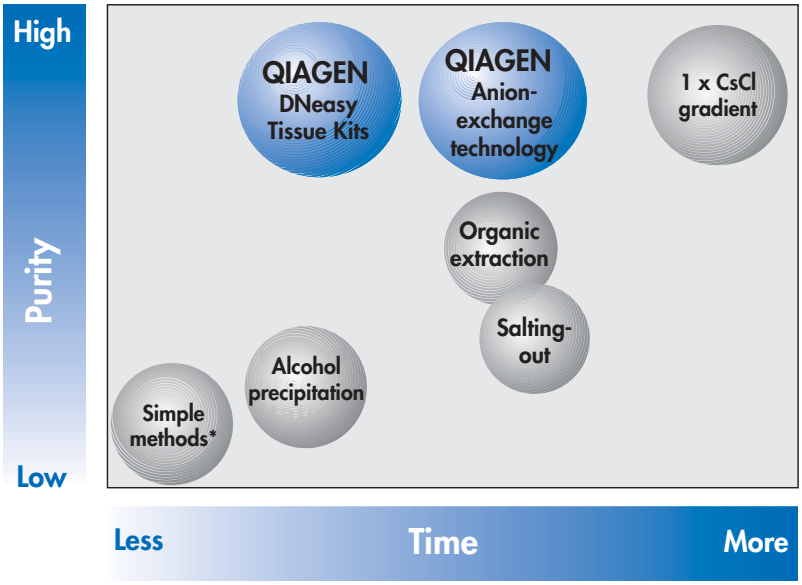

Figure 7. *Length of time taken for the common methods of genomic DNA isolation, after proteinase K digestion. Simple methods are defined as boiling preps and other protocols that do not include a lysis step. Alcohol precipitation is the precipitation of proteinase K-digested lysates after removal of insoluble particles by centrifugation. For organic extraction phenol/chloroform is added to the proteinase K-digested lysate, vortexed and centrifuged. The upper phase is then subject to alcohol precipitation. Salting-out is defined as treatment of the proteinase K-digested lysate with a high-salt buffer. This is incubated and the proteins precipitated by centrifugation. The supernatant is then subject to alcohol precipitation. See pages 10–12 for further information on DNA isolation methods.*

** No proteinase K digestion.*