Purification of DNA by Anion-Exchange Chromatography

Column chromatography has recently evolved to provide a rapid and effective alternative to more laborious methods for preparing high-quality DNA, such as CsCl-gradient centrifugation. This protocol describes the use of a column made of a unique anion-exchange resin that selectively binds nucleic acids, allowing rapid separation of DNA from contaminating RNA, proteins, carbohydrates, and metabolites. The procedure below employs columns supplied by QIAGEN; other preparation methods are available from other suppliers. A crude nucleic acid sample (usually a cleared cell lysate) is applied to the QIAGEN-tip under conditions that favor binding. Contaminants in the sample are washed from the column with a moderate-salt buffer, and DNA is eluted using a high-salt buffer.

Materials

- Plasmid- or phage-bearing bacterial culture or mammalian, plant, or bacterial cell culture
- Buffer QBT (equilibration buffer; see recipe)
- QIAGEN-tip of appropriate size (Table 2.1B.1)
- Buffer QC (washing buffer; see recipe)
- Buffer QF (eluting buffer; see recipe)
- TE buffer, pH 8.0 (APPENDIX 2)
- Isopropanol, room temperature
- 70% (v/v) ethanol, ice cold
- Beckman JS-13, Sorvall HB-4 or HB-6, or equivalent rotor

Additional reagents and equipment for preparation of mammalian, plant, or bacterial genomic DNA (cell lysate; UNITS 2.2-2.4), alkaline lysis preparation of plasmid DNA (UNIT 1.7), or preparation of phage lysates (UNIT 1.13)

1. To isolate plasmid, cosmid, or phage DNA, prepare a cell lysate from a strain harboring the appropriate vector. To isolate genomic DNA, prepare cell lysate from mammalian, plant, or bacterial culture. Remove a sample from the cleared lysate and

<table>
<thead>
<tr>
<th>Table 2.1B.1</th>
<th>Recommended Loading Volumes, DNA Capacities, and Culture Volumes for QIAGEN-tips</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>QIAGEN-tip 100</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Column DNA capacity</th>
<th>QIAGEN-tip 100</th>
<th>QIAGEN-tip 500</th>
<th>QIAGEN-tip 2500</th>
<th>QIAGEN-tip 10000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmid or ds phage</td>
<td>100 µg</td>
<td>500 µg</td>
<td>2500 µg</td>
<td>10000 µg</td>
</tr>
<tr>
<td>Cosmid or λ phage</td>
<td>60 µg</td>
<td>300 µg</td>
<td>1500 µg</td>
<td>6000 µg</td>
</tr>
<tr>
<td>Mammalian or B. subtilis genomic</td>
<td>80 µg</td>
<td>400 µg</td>
<td>2000 µg</td>
<td>8000 µg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Culture volume for plasmid DNA</th>
<th></th>
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<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>High-copy-number plasmid</td>
<td>25 ml</td>
<td>100 ml</td>
<td>500 ml</td>
<td>2.5 liters</td>
</tr>
<tr>
<td>Low-copy-number plasmid</td>
<td>100 ml</td>
<td>500 ml</td>
<td>2.5 liters</td>
<td>5 liters</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Culture volume for genomic DNA</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis</td>
<td>8 ml</td>
<td>40 ml</td>
<td>200 ml</td>
<td>800 ml</td>
</tr>
<tr>
<td>Mammalian (no. cells)</td>
<td>$2 \times 10^7$</td>
<td>$1 \times 10^8$</td>
<td>$5 \times 10^8$</td>
<td>$2 \times 10^9$</td>
</tr>
</tbody>
</table>

Preparation and Analysis of DNA
save for an analytical gel to determine whether growth and lysis conditions were optimal.

*For plasmid preps, lysozyme treatment is not necessary. Plasmid and phage preps should include RNase A treatment, because the long RNA molecules can compete with DNA for binding sites on the resin. When the sample is loaded on the QIAGEN-tip, it must have a salt concentration of 750 mM and a pH of 7.0, and should be free of particulate matter, which will clog the column.*

*Bacterial lysates can be cleared by a conventional centrifugation or by filtration through a special filter unit available from QIAGEN. The QIAClean filter is available in syringe or cartridge format and is suitable for small to large bacterial culture volumes. It has been designed to rapidly clear bacterial lysates and completely remove SDS precipitates without centrifugation.*

*For endotoxin-free DNA, QIAGEN offers EndoFree Plasmid Kits containing an endotoxin-removal buffer that is briefly incubated with the cleared lystate prior to loading on the QIAGEN-tip. Plasmid DNA purified using EndoFree Plasmid Kits contains only negligible amounts of endotoxin.*

Anionic detergents such as SDS will inhibit DNA binding to the resin; thus, their use in preparing these cell lysates should be avoided if possible (see Tables 10.15.1 and 10.15.2 for physical and chemical properties of commonly used detergents). If anionic detergents must be included in any of the extraction or lysis buffers, they may be removed by precipitation with acidic potassium acetate (UNIT 1.7) followed by centrifugation. Small SDS precipitates that cannot be separated by conventional centrifugation are completely removed by the QIAfilter process.

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2. Equilibrate an appropriately sized QIAGEN-tip with Buffer QBT by applying 2 bed volumes of buffer and allowing it to empty by gravity flow.

*The flow of buffer will stop when the meniscus reaches the upper column frit. Do not force out the remaining buffer.*

3. Apply cleared lysate from step 1 to the equilibrated column and allow it to enter the resin by gravity flow. Remove a small sample of flowthrough (50 to 500 µl, depending on the column size) and save for analytical gel electrophoresis.

4. Wash column twice with 3 to 6 bed volumes of Buffer QC (depending on column size) and allow the buffer to move through the QIAGEN-tip by gravity flow. Remove a small sample of flowthrough and save for analytical gel electrophoresis.

*This step removes cellular contaminants and any remaining RNA from the DNA.*

5. Elute pure DNA by applying 1 to 2 bed volumes of Buffer QF (depending on column size) and allowing it to flow through by gravity flow. Remove a small sample of eluate and save for analytical gel electrophoresis.

6. Add 0.7 vol isopropanol to eluate and immediately centrifuge 30 min at 15,000 × g (≥9500 rpm in Beckman JS-13), 4°C, to precipitate DNA. Remove and discard supernatant.

7. Wash pellet with ice-cold 70% ethanol, air dry 10 min, and redissolve in a suitable volume of TE buffer, pH 8.0.

*Overdrying will make the pellet difficult to dissolve. Do not pipet high-molecular-weight DNA up and down when dissolving, as this may cause shearing.*

8. **Optional:** To analyze the purification procedure, precipitate the samples taken in steps 1, 3, 4, and 5 with 0.7 vol isopropanol. Rinse pellets with 70% ethanol, drain well, and resuspend in 10 µl TE buffer, pH 8.0. Use 2 µl of each for analysis on a 1% agarose gel (UNIT 2.5A; see Critical Parameters and Troubleshooting).
REAGENTS AND SOLUTIONS

Use deionized, distilled water in all recipes and protocol steps. For common stock solutions, see APPENDIX 2; for suppliers, see APPENDIX 4.

**Buffer QBT**

- 750 mM NaCl
- 50 mM 3-(N-morpholino)propanesulfonic acid (MOPS)
- Adjust pH to 7.0 with HCl
- 15% (v/v) isopropanol
- 0.15% (v/v) Triton X-100
- Store indefinitely at room temperature

**Buffer QC**

- 1.0 M NaCl
- 50 mM MOPS
- Adjust pH to 7.0 with HCl
- 15% (v/v) isopropanol
- Store indefinitely at room temperature

**Buffer QF**

- 1.25 M NaCl
- 50 mM Tris·Cl
- 15% isopropanol
- Adjust pH to 8.5 with HCl
- Store indefinitely at room temperature

COMMENTARY

**Background Information**

For many routine applications in molecular biology, DNA used as starting material must be very pure. Until recently, traditional CsCl/ethidium bromide gradients (for plasmid and phage DNA; UNITS 1.7 & 1.13) or organic extraction (for genomic DNA; UNIT 2.1A) have been widely used. Although they provide a highly purified product, these methods are generally time-consuming, may involve the use of hazardous amounts of toxic reagents, and do not yield endotoxin-free plasmid DNA. A simple and time-saving alternative to these methods, without sacrifice in quality, is provided by ion-exchange chromatography. This technique was originally used for protein purification (see UNIT 10.10 for a complete discussion), but is now also widely used for nucleic acid separation.

The general principles of ion-exchange purification hold true for nucleic acid separations: negatively charged nucleic acids are applied to an oppositely charged chromatographic matrix. The best choice for these separations is an anion-exchange resin carrying positively charged groups that adsorb negatively charged molecules in buffers near neutral pH and of medium ionic strength. In particular, the high surface density of anion-exchange (DEAE) groups—together with the large pore size of the commercially available QIAGEN Anion-Exchange Resin—permits attachment and retention of the highly charged nucleic acid molecules on the column. Subsequent washing of the column to remove impurities contained in the lysate results in a very high degree of purification of the DNA.

The separation range of conventional anion exchangers for nucleic acids extends only up to ~0.5 M salt. Because binding and elution of the components of a lysate are limited to a narrow range of salt concentrations, the elution peaks of proteins, RNA, and DNA overlap with one another, preventing effective separation of DNA from contaminants. Because of the extremely high charge density (QIAGEN Resin contains ~5-fold more DEAE groups per unit surface area of resin than do conventional anion exchangers), the resin described here separates various nucleic acid forms over a broader range of salt concentration (up to 1.6 M, the elution point for double-stranded DNA; Fig. 2.1B.1). Because the charge density of the resin is inversely proportional to the pH of the buffer, the binding, washing, and elution profiles are strongly affected by the pH of the relevant buffers (Fig. 2.1B.2).
Anion-exchange methods have been used in conjunction with a variety of applications whose success depends critically upon the purity of the nucleic acid sample, including transfection of mammalian cells (Chen et al., 1991), DNA sequencing (Hall et al., 1989; Voss et al., 1990), PCR (Jones and Winistorfer, 1992; Jung et al., 1992), and runoff transcription (Kayne et al., 1988; Luytjes et al., 1989). In addition, the method has been used to recover plasmid and viral DNA from mammalian cells (Lutze and Winegar, 1990).

Further alternatives to traditional purification procedures include gel filtration as well as

![Figure 2.1B.1](image-url)  
*Figure 2.1B.1* Separation of nucleic acids at neutral pH on QIAGEN Anion-Exchange Resin (reproduced with permission from QIAGEN, 1997).

![Figure 2.1B.2](image-url)  
*Figure 2.1B.2* Elution points of different nucleic acids from QIAGEN Anion-Exchange Resin as a function of pH (reproduced with permission from QIAGEN, 1997).
<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible cause(s)</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>No DNA in sample lysate before loading</td>
<td>Plasmid failed to propagate</td>
<td>Check that conditions for optimum plasmid growth were met</td>
</tr>
<tr>
<td></td>
<td>Lysate was incorrectly prepared</td>
<td>Check age of buffers and prepare fresh if necessary</td>
</tr>
<tr>
<td>DNA in flowthrough fraction</td>
<td>Column overloaded with DNA</td>
<td>Check yield against capacity of column (see Table 2.1B.1). Purify excess DNA by passing through new tip.</td>
</tr>
<tr>
<td></td>
<td>SDS or other ionic detergent in the DNA sample</td>
<td>Ensure that SDS is removed from lysate before column loading by dialyzing or precipitating with cold potassium acetate</td>
</tr>
<tr>
<td>High-molecular-weight RNA in eluate</td>
<td>RNase A digestion insufficient</td>
<td>Check culture volume against recommended volumes in Table 2.1B.1 and reduce if necessary. Check effectiveness of RNase A solution. Recover DNA by precipitating the eluate, digesting with RNase A, and purifying on a new tip</td>
</tr>
<tr>
<td>DNA found in wash fraction</td>
<td>Column overloaded with DNA</td>
<td>Check the culture volume and yield against the capacity of the respective QIAGEN-tip. Reduce culture volume accordingly.</td>
</tr>
<tr>
<td></td>
<td>Wash buffer incorrect</td>
<td>Check pH and salt concentration of Buffer QC. Recover DNA by precipitation and purify on another column.</td>
</tr>
<tr>
<td>No DNA in eluate</td>
<td>Elution buffer incorrect</td>
<td>Check pH and salt concentration of Buffer QF. Recover DNA by eluting with fresh buffer.</td>
</tr>
<tr>
<td>Little or no DNA upon precipitation</td>
<td>DNA failed to precipitate</td>
<td>Check isopropanol batch. Make sure that precipitate is centrifuged at &gt;15,000 × g for 30 min; if necessary, centrifuge longer at higher speeds. Mark expected location of pellet before centrifugation</td>
</tr>
<tr>
<td></td>
<td>DNA pellet lost (isopropanol pellets are glassy and may be difficult to see)</td>
<td>DNA poorly resuspended</td>
</tr>
<tr>
<td>DNA difficult to resuspend</td>
<td>Pellet overdried</td>
<td>Air dry pellet; do not use a vacuum. Warm DNA solution slightly to dissolve. Residual isopropanol in pellet Too much salt in pellet</td>
</tr>
<tr>
<td>RNA in eluate</td>
<td>RNase A digestion insufficient</td>
<td>Check culture volume against recommended volumes in Table 2.1B.1 and reduce if necessary. Increase volume of wash buffer, recover DNA by precipitating eluate, digest with RNase A, and purify.</td>
</tr>
<tr>
<td>Genomic DNA contamination in eluate (for plasmid preps)</td>
<td>Mixing too vigorous</td>
<td>Lysate must be handled gently to prevent shearing. Reduce culture volume if lysate is too viscous for gentle mixing. Lysis time too prolonged</td>
</tr>
</tbody>
</table>

*continued*
additional separation methods (e.g., G-25/G-50, Select, and pZ spin columns, available from 5 Prime → 3 Prime, and Wizard DNA purification resin, available from Promega; also see UNIT 1.13). Gel-filtration columns purify DNA by allowing large nucleic acid molecules to pass through while retarding the migration of contaminants (small RNAs and oligonucleotides) through the gel. In general, these alternatives are comparable in terms of the purity of the sample obtained, although some may permit carryover of residual impurities from the starting sample. Opinions vary, however, regarding the relative values of the different methods. For example, recent reports assert that plasmid DNA purified by QIAGEN Anion-Exchange Resin is at least comparable (and may be superior) to preparations purified using CsCl. Determinations were made by measuring transfection efficiencies (Ehlert et al., 1993) and by observations of plasmid preparations using electron microscopy (Schleef and Heilmann, 1993). In a series of biological assays, various purified preparations of plasmid DNA injected into Drosophila embryos were subsequently analyzed for the ability to generate germline-transformed flies. No significant differences were observed among CsCl-, PEG-, QIAGEN-, or pZ-purified plasmids, although PEG-derived preparations appeared to be slightly less toxic to the cells (Joseph Heilig, pers. commun.).

Critical Parameters and Troubleshooting

QIAGEN Anion-Exchange Resin has different binding capacities for different classes of nucleic acids: the binding capacity for RNA, for example, is roughly twice that for plasmid DNA. Larger DNA molecules (e.g., phage, cosmids, BACs, P1s, and genomic DNAs) are bound at an even lower capacity than plasmid DNA. Therefore, when selecting a column size, the type of nucleic acid to be purified should be taken into account. It is also important to choose a column with the appropriate binding capacity for the culture volume used, to avoid overloading (see Table 2.1B.1). For plasmid, phage, and cosmids preparations, this is achieved by careful control of culture conditions and inclusion of an RNase treatment in the lysate preparation. While the use of super-rich medium such as Superbroth (UNIT 1.1) or Terrific broth (Tartoff and Hobbs, 1987; UNIT 1.7) can be advantageous for culturing cosmids and plasmids maintained at low copy number, high-copy-number plasmids do not require growth in super-rich medium to give a reasonable yield. In these cases, use of super-rich medium can result in extraordinarily high cell densities with a corresponding increase in lysate viscosity due to the presence of large amounts of nucleic acid or protein. Before loading the column, ensure that the lysate is clear and free of particulate matter and adjust the loading volume as indicated in Table 2.1B.1.

Table 2.1B.2 Troubleshooting Guide for DNA Purification by Anion-Exchange Chromatography (adapted from QIAGEN, 1997), continued

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible cause(s)</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA is nicked, sheared, or degraded</td>
<td>Endonuclease-containing host</td>
<td>Consider changing E. coli host strain</td>
</tr>
<tr>
<td></td>
<td>Nuclease contamination present</td>
<td>Check buffers for nuclease contamination and replace if necessary. Use autoclaved glass and plastic and wear gloves.</td>
</tr>
<tr>
<td></td>
<td>DNA poorly buffered</td>
<td>Resuspend DNA in TE, pH 8.0, to inhibit nuclease activity and maintain stable pH during storage</td>
</tr>
<tr>
<td></td>
<td>Shearing during resuspension</td>
<td>Resuspend DNA gently without vortexing or vigorous pipetting</td>
</tr>
<tr>
<td>DNA does not perform well in later experiments</td>
<td>DNA nicked</td>
<td>Check percentage of nicked DNA on agarose gel</td>
</tr>
<tr>
<td></td>
<td>Too much salt</td>
<td>Make sure isopropanol is at room temperature for precipitation and pellet is washed with 70% ethanol</td>
</tr>
<tr>
<td></td>
<td>Residual protein</td>
<td>Check culture volume against recommended volumes in Table 2.1B.1 and reduce if necessary. Increase volume of wash buffer.</td>
</tr>
</tbody>
</table>
Another critical factor is the pH of the column buffers used for binding, washing, and eluting (Fig. 2.1B.2). For optimal recovery, the final pH should be adjusted as recommended in the recipes (see Reagents and Solutions); changes in pH may lead to loss of DNA.

Many factors may cause low or no yield or incomplete purification of DNA. Checking an aliquot of each fraction on an analytical gel (Fig. 2.1B.3) can help locate the source of the problem. Table 2.1B.2 describes various problems that may be encountered and provides suggestions for how to deal with them.

**Anticipated Results**

The expected yield for a preparation of genomic DNA from mammalian cell culture is ~30 to 40 µg per 10^7 cells. Low-copy-number plasmid and cosmid preparations from cells grown overnight in LB medium yield 0.2 to 1 µg DNA per milliliter of overnight culture, while high-copy-number plasmids prepared under these conditions yield 3 to 5 µg/ml. When grown in super-rich medium, the yield of cosmids and low-copy-number plasmids is increased to 2.5 to 5 µg DNA/ml, and that of high-copy-number plasmids to 5 to 25 µg/ml.

**Time Considerations**

With most types of cells, preparing the original DNA sample (lysate) and running the column (including equilibration time, sample loading, washing, and elution) should take 1.5 to 4 hr, depending upon the volume of lysate and the corresponding column size.

**Literature Cited**


**Figure 2.1B.3**  Agarose analytical gel (1%) comparing DNA composition of QIAGEN-tip elution fractions at different stages of plasmid purification (lanes 2 to 6) or containing different types of plasmid DNA (lanes 7 to 11). Lanes: 1, lambda *HindIII* marker; 2, cleared lysate before column purification; 3, flowthrough fraction; 4,5, first and second Buffer QC washes; 6, eluted plasmid DNA; 7, fraction containing denatured supercoiled DNA; 8, fraction containing multimeric forms of supercoiled plasmid DNA; 9, fraction containing linear plasmid DNA (*pTZ19/EcoR*I); 10, fraction contaminated with bacterial chromosomal DNA; 11, fraction 10 digested with *EcoR*I; 12, lambda *HindIII* marker. (reproduced with permission from QIAGEN, 1997).


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