

SOP-105

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# Plasmid DNA

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Procedures for DNA gel electrophoresis.

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Gel Electrophoresis Unit

UV Transilluminator & UV Eye Protection

Photo Documentation Equipment

Electrophoresis Buffer (TAE)

50X stock solution pH ~8.5

242 g Tris base

57.1 ml glacial acetic acid

37.2 g  $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$

Add distilled water to 1 liter, make 0.5 ug / ml Ethidium bromide

DNA Ladder

1 kb DNA ladder (New England BioLabs)

Agarose

10X Loading Buffer

20% (w/v) Ficoll 400

0.1 M  $\text{Na}_2\text{EDTA}$ , pH 8.0

1.0% SDS

0.25% bromphenol blue

0.25% xylene cyanol

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### Agarose Gel Analysis of Plasmid DNA

The main uses of agarose gels for plasmid DNA analysis are:

1. Analysis of the size and conformation of nucleic acids in a sample
2. Quantification of DNA
3. Separation and extraction of DNA fragments

### Procedure

1. Prepare gel using electrophoresis buffer and electrophoresis grade agarose. Effective visualization of plasmid DNA can be performed using appropriate concentration of agarose. **Note: Buffer contains ethidium bromide which is toxic. Wear lab coats and gloves during preparation.**

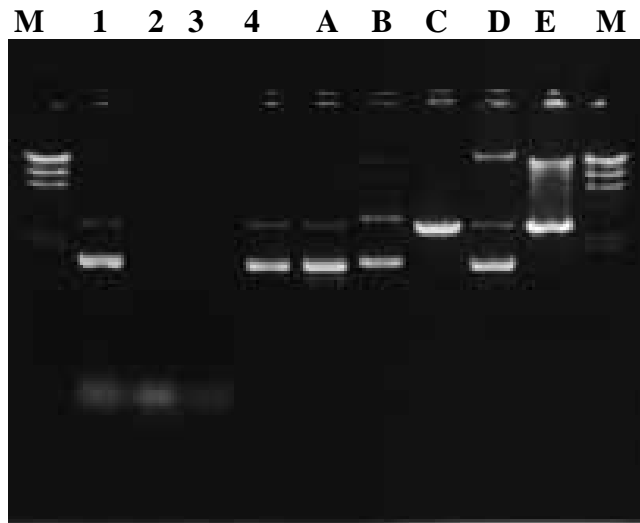
**Table 1. Suggested Agarose Concentration**

% Agarose	Effective Range of DNA Fragments (kb)
0.5	30 - 1
0.7	12 – 0.8
1.0	10 – 0.5
1.2	7 – 0.4
1.5	3-0.2

2. Melt agarose gel in microwave. Allow to cool to ~55C and then pour into electrophoresis casting unit. Place comb into casting unit to form sample wells. After gel has hardened place into electrophoresis unit. Cover gel with 1 x electrophoresis buffer.
3. Prepare DNA samples with appropriate amount of 10 loading buffer. Load samples into wells with pipettor.
4. Apply current to electrophoresis unit: 1-10V / cm of gel.
5. Turn off power when bromophenol blue has reached bottom of gel.
6. Photograph gel directly on UV transilluminator. **Wear UV eye protection.**

## Analysis of a purification procedure

Image taken from Qiagen, [Plasmid DNA: Agarose gel analysis of plasmid DNA - English \(PDF\)](#).



**Figure 1** Shows how agarose gel electrophoresis can be used to analyze the nucleic acid content of samples taken during a plasmid purification procedure. The gel demonstrates successful plasmid purification using anion-exchange columns as well as some atypical results.

**M:** Lambda DNA digested with *HindIII*.

**1:** Cleared lysate containing supercoiled (lower band) and open circular plasmid DNA (upper band) and degraded RNA (smear at the bottom of the gel).

**2:** Flow-through fraction containing only degraded RNA (the plasmid DNA is bound to the anion-exchange resin in the column).

**3:** Wash fraction to ensure that the resin in the column is cleared of RNA and other contaminants (plasmid DNA remains bound to the column).

**4:** Elution containing pure plasmid DNA in supercoiled and open circular forms.

Lanes A–E illustrate some atypical results that may be observed in some preparations, depending on plasmid type and host strain.

**A:** Supercoiled (lower band) and open circular form (upper band) of the high-copy plasmid, pUC18, with an additional band of denatured supercoiled DNA migrating just beyond the supercoiled form.

**B:** Multimeric forms of supercoiled plasmid DNA (pTZ19) that may be observed with some host strains and should not be mistaken for genomic DNA. Multimeric plasmid DNA is easily distinguished from genomic DNA by restriction digestion.

**C:** Linearized form of plasmid pTZ19 after restriction digestion with *Eco*RI.

**D:** Sample contaminated with bacterial chromosomal DNA (uppermost band).

**E:** *Eco*RI digestion of a sample contaminated with bacterial genomic DNA, which gives a smear above the plasmid DNA.

With large-constructs such as BAC, PAC, and P1 DNA, the supercoiled form migrates at a slower rate than the linear form. Furthermore, large-construct DNA >50 kb is often difficult to distinguish from genomic DNA by agarose gel analysis.

### Gel extraction

Agarose gels can be used for separation and extraction of DNA fragments, for example, a specific DNA fragment from a PCR or restriction digestion reaction. Ensure that the percentage of agarose used for the gel allows good separation of DNA fragments for easy excision. Run agarose gels for DNA extraction at a low voltage. This will enable efficient separation of DNA bands without smearing, facilitating excision of the gel slice. Excise the fragment quickly under low-strength UV light to limit DNA damage.

DNA fragments can be extracted quickly and efficiently from agarose gels using silica-gel-based purification. Silica-gel-based methods typically result in higher and more reproducible recoveries than other gel extraction methods, such as electroelution, and require no phenol extraction or ethanol precipitation. In a typical silica-gel-based purification procedure, the agarose gel slice is first solubilized. DNA is then bound to the silica-gel material in the presence of high concentrations of chaotropic salts. A wash step removes impurities, and DNA is then eluted in low-salt buffer.

### Spectrophotometry

DNA concentration can be determined by measuring the absorbance at 260 nm ( $A_{260}$ ) in a spectrophotometer using a quartz cuvette. For greatest accuracy, readings should be between 0.1 and 1.0. An absorbance of 1 unit at 260 nm corresponds to 50  $\mu$ g genomic DNA per ml ( $A_{260} = 1 = 50 \mu\text{g/ml}$ ). This relation is valid only for measurements made at neutral pH, therefore, samples should be diluted in a low-salt buffer with neutral pH (e.g., Tris·Cl, pH 7.0).

If you will use more than one cuvette to measure multiple samples, the cuvettes must be matched. Spectrophotometric measurements do not differentiate between DNA and RNA, so RNA contamination can lead to overestimation of DNA concentration. Phenol has an absorbance maximum of 270–275 nm, which is close to that of DNA. Phenol contamination mimics both higher yields and higher purity, because of an upward shift in the  $A_{260}$  value.

### Purity of DNA

The ratio of the readings at 260 nm and 280 nm ( $A_{260}/A_{280}$ ) provides an estimate of DNA purity with respect to contaminants that absorb UV light, such as protein. The  $A_{260}/A_{280}$  ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting  $A_{260}/A_{280}$  ratio can vary greatly. Lower pH results in a lower  $A_{260}/A_{280}$  ratio and reduced sensitivity to protein contamination. For accurate  $A_{260}/A_{280}$  values, we recommend measuring absorbance in a slightly alkaline buffer (e.g., 10 mM Tris·Cl, pH 7.5). Make sure to zero the spectrophotometer with the appropriate buffer. Pure DNA has an  $A_{260}/A_{280}$  ratio of 1.7–1.9. Scanning the absorbance from 220–320 nm will show whether there are contaminants affecting absorbance at 260 nm. Absorbance scans should show a peak at 260 nm and an overall smooth shape. Phenol has an absorbance maximum of 270–275 nm, which is close to that of DNA. Phenol contamination mimics both higher purity and higher yields, because of an upward shift in the  $A_{260}$  value.

## Section

# 3

## References

Short Protocols in Molecular Biology. 4<sup>th</sup> Edition. Editors Frederick M. Ausubel, Roger Brent, Robert E. Kingston, David D. Moore, J.G. Seidman, John A Smith and Kevin Struhl. John Wiley & Sons, Inc. 1999

<http://www.qiagen.com/> :

[Plasmid DNA: Agarose gel analysis of plasmid DNA - English \(PDF\)](#)

<http://www.neb.com>