Genetic Tools and Reagents

Bacterial Culture Media, Agar, Yeast Extract, Casein Peptone
Plasmid Purification

Bacterial Culture & Plasmid Purification

Product Manual

Bacterial Culture Media Protocols & Principle
Plasmid Purification Protocols & Principle

Gene Link™

Ordering Information

Bacterial Culture Media		
Product	**Catalog No.**	**Unit Size**
Agar Type A Bacterial Culture Grade, 100 g	40-3301-10	100 g
Agar Type A Bacterial Culture Grade, 500 g	40-3301-05	100 g
Agar Type A Bacterial Culture Grade, 1 kg	40-3301-01	1 kg
Yeast Extract Bacterial Culture Grade, 100 g	40-4331-10	100 g
Yeast Extract Bacterial Culture Grade, 500 g	40-4331-10	500 g
Yeast Extract Bacterial Culture Grade, 1 k g	40-4331-10	1 kg
Casein Peptone (Type 1) Bacterial Culture Grade, 100 g	40-3305-10	100 g
Casein Peptone (Type 1) Bacterial Culture Grade, 500 g	40-3305-05	500 g
Casein Peptone (Type 1) Bacterial Culture Grade, 1 kg	40-3305-01	1 kg

Omni-Pure™ Plasmid DNA Purification Systems; Mini-Prep		
Product	**Catalog No.**	**Unit Size***
Omni-Pure™ Plasmid DNA Purification System; Mini-Prep	40-4020-01	100
Omni-Pure™ Plasmid DNA Purification System; Mini-Prep	40-4020-05	500
*Mini-prep plasmid purification.		

Bacterial Culture & Plasmid Purification Protocols & Principle

Contents

Bacterial Culture Media Protocols & Principle

Agar Type A Bacterial Culture Grade		
Product	**Catalog No.**	**Unit Size**
Agar Type A Bacterial Culture Grade, 100 g	40-3301-10	100 g
Agar Type A Bacterial Culture Grade, 500 g	40-3301-05	100 g
Agar Type A Bacterial Culture Grade, 1 kg	40-3301-01	1 kg

Personal Protective Equipment for Handling: Eye shields & Type P1 (EN143) respirator filter.
To protect from product dust and mists a respirator is strongly advised during handling.

Product Description & Application

Agar Type A Bacterial Culture Grade is obtained from certified supplier as pharmaceutical grade product that underwent quality control tests as listed below. This product is qualified for routine bacterial culture.

This type of agar is used as a solidifying agent in culture media. Its main characteristic is the complete absence of inhibitors, which could otherwise hinder growth of microorganisms. Bacteriological agar also has excellent transparency, high hysteresis (difference between melting and gelling temperatures) and superior batch to batch consistency

Synonym: Agar-agar, Agar, Gum agar

CAS Number: 9002-18-0

Linear Formula: $(C_{12}H_{18}O_9)_n$

EC Number: 232-658-1

Technical Data	Specifications
Color	White Cream
Odor	Nil
Particle Size	60 mesh: 95% pass
Moisture	10.14%
pH (1.5% solution nt 60oC)	7.18%
Water Absorption	49 c.c.
Solubility	Boiling Water
Gel Strength (method Nikkan 1.5% at 20°C)	880 gr/cm^2
Clarity	Up 25 cm
Melting Point	92°C
Gelling Point	36°C
Moulds and yeast	<100/g.
E. Coli	Nil
Coliforms	<3/g.
Source of Material	Seaweed. It contains no animal components

Yeast Extract Bacterial Culture Grade		
Product	**Catalog No.**	**Unit Size**
Yeast Extract Bacterial Culture Grade, 100 g	40-4331-10	100 g
Yeast Extract Bacterial Culture Grade, 500 g	40-4331-10	500 g
Yeast Extract Bacterial Culture Grade, 1 k g	40-4331-10	1 kg

Personal Protective Equipment for Handling: Eye shields & Type P1 (EN143) respirator filter.

To protect from product dust and mists a respirator is strongly advised during handling.

Yeast Extract Bacterial Culture Grade

Product Description & Application

Yeast extract is manufactured under controlled conditions to retain its vitamin content and other nutritive values such as free amino acids. It is rich in vitamins, especially those belonging to B complex and is often used to supply these factors in culture media at a concentration of 0.3 - 0.5%.

Yeast Extract Bacterial Culture Grade is obtained from certified supplier as product that underwent quality control tests as listed below. This product is qualified for routine bacterial culture.

Chemical Characteristics	**Typical Specifications**
Amino Nitrogen (AN)	5.30%
Total Nitrogen (TN)	11.10%
pH (2% solution)	6.8
Growth Supporting Properties	
Peptone agar	Satisfactory
Broth Formulations	Satisfactory
Microbiological Analysis	
Standard plate count	Less than 5000 col/g
Yeasts and molds	Less than 100 col/g
Coliforms	Negative
Salmonella	Negative
E. coli	Negative

Casein Peptone (Type 1) Bacterial Culture		
Product	**Catalog No.**	**Unit Size**
Casein Peptone (Type 1) Bacterial Culture Grade, 100 g	40-3305-10	100 g
Casein Peptone (Type 1) Bacterial Culture Grade, 500 g	40-3305-05	500 g
Casein Peptone (Type 1) Bacterial Culture Grade, 1 kg	40-3305-01	1 kg

Personal Protective Equipment for Handling: Eye shields & Type P1 (EN143) respirator filter.

To protect from product dust and mists a respirator is strongly advised during handling.

Casein Peptone (Type 1) Bacterial Culture Grade Product Description & Application

Casein Peptone (Type 1) Bacterial Culture Grade is an enzymatic tryptic digest of casein. It contains all the amino acids found in casein as well as larger peptide fractions. It is an excellent nutrient for use in culture media for producing antibiotics, toxins, enzymes and other biological products. This product is widely used in the pharmaceutical and veterinary industries and in diagnostic culture media.

Chemical Characteristics	**Typical Analysis**
Amino Nitrogen (AN)	3.90%
Total Nitrogen (TN)	12.20%
Loss on drying	3.30%
Ash	7.20%
pH (2% solution)	7.3
Undigested proteins	negative
Growth Supporting Properties	
Peptone agar	Satisfactory
Broth Formulations	Satisfactory
Microbiological Analysis	
Standard plate count	Less than 5000 CFU/g
Yeasts and molds	Less than 100 CFU/g
Coliforms	Negative
Salmonella	Negative

Plasmids, Host Strains and Propagation

Plasmid Copy Number

Plasmids vary widely in their copy number depending on the origin of replication they contain (e.g. pMB1 or pSC101) which determines whether they are under relaxed or stringent control; as well as the size of the plasmid and its associated insert. Some plasmids, such as the pUC series and derivatives, have mutations, which allow them to reach very high copy numbers within the bacterial cell. Plasmids based on pBR322 and many cosmids are generally maintained at lower copy numbers. Very large plasmids are often maintained at very low copy numbers per cell. The copy number of plasmids and cosmids can be substantially influenced by the cloned insert. For example, a high-copy pUC plasmid may behave like a medium or low-copy plasmid when containing certain inserts (e.g. very large DNA fragments resulting in lower DNA yields than expected.

- pSC101 and pBR322 are low copy number plasmids.
- pUC, pBluescript and pGem series vectors are high copy number.

Origins of replication and copy numbers of various plasmids and cosmids

Plasmids	Origin of replication	Copy number	Plasmid Copy
pUC vectors	pMB1*	500–700	high copy
pBluescript® vectors	ColE1	300–500	high copy
pGEM® vectors	pMB1*	300–400	high copy
pTZ vectors	pMB1*	>1000	high copy
pBR322 and derivatives	pMB1*	15–20	low copy
pACYC and derivatives	p15A	10–12	low copy
pSC101 and derivatives	pSC101	~5	very low copy
Cosmids			
SuperCos	ColE1	10–20	low copy
pWE15	ColE1	10–20	low copy

** The pMB1 origin of replication is closely related to that of ColE1 and falls in the same incompatibility group. The high-copy plasmids listed here contain mutated versions of this origin.*

Recipe

LB Broth	
An all purpose media for the growth of bacterial culture	
Tryptone (casein peptone)	10.0 g/L
Yeast Extract	5.0 g/L
NaCl	10.0 g/L

LB Agar Plates	
An all purpose media for the growth of bacteria on plates	
Tryptone (casein peptone)	10.0 g/L
Yeast Extract	5.0 g/L
NaCl	10.0 g/L
Agar	15.0 g/L

Terrific Broth	
Highly enriched culture media for improved yield of plasmid DNA	
Tryptone (casein peptone)	12.0 g/L
Yeast Extract	24.0 g/L
K_2HPO_4	9.4 g/L
KH_2PO_4	2.2 g/L
Sterilize and then add 8ml/L glycerol	

SOB	
Media for competent cell manipulation prior to transformation	
Tryptone (casein peptone)	20.0 g/L
Yeast Extract	5.0 g/L
NaCl	0.50 g/L
$MgSO_4$	5.0 g/L

Culture media

Traditionally the standard Luria Bertani (LB) media is used to grow bacterial cell. The Omni-Pure ™ plasmid purification system is optimized for use with LB media. We advise harvesting cultures after approximately 12–16 hours of growth, which typically is the transition from logarithmic into stationary growth phase. At this time, the ratio of plasmid DNA to RNA is higher than during the logarithmic phase. Also, the DNA is not yet degraded due to over aging of the culture, as in the later stationary phase. Please note the maximum recommended culture volumes given at the beginning of each protocol.

Host strains

Most *E. coli* strains can be used successfully to isolate plasmid DNA, although the strain used to propagate a plasmid can have a substantial influence on the quality of the purified DNA. Host strains such as DH1, DH5α™, and C600 yield high-quality DNA. The slower growing strain XL1-Blue also yields DNA of very high quality, which works extremely well for sequencing. Strain HB101 and its derivatives, such as TG1 and the JM100 series, contain large amounts of carbohydrates that are released during lysis and can inhibit enzyme activities if not completely removed (3). In addition, some strains such as JM101, JM110, and HB101 have high levels of endonuclease activity, and yield DNA of lower quality than that prepared from strains such as XL1-Blue, DH1, DH5α, and C600. The methylation and growth characteristics of the host strain can also affect plasmid isolation.

- Host Strain JM101 series and HB101 have high levels of carbohydrate and endonuclease.
- DH1, DH5α™ and XL1 blue yield high quality plasmid DNA.

Inoculation

Bacterial cultures for plasmid preparation should always be grown from a single colony picked from a freshly streaked selective plate. Sub culturing directly from glycerol stocks, agar stabs, and liquid cultures is poor microbiological practice and may lead to loss of the plasmid. Inoculation from plates that have been stored for a long time may also lead to loss or mutation of the plasmid.

The desired clone should be streaked from a glycerol stock onto a freshly prepared agar plate containing the appropriate selective agent such that single colonies can be isolated. This procedure should then be repeated to ensure that a single colony of an antibiotic-resistant clone can be picked. A single colony should be inoculated into 2–10 ml of LB medium containing the appropriate selective agent and grown for 12-16 hours with vigorous shaking (~300 rpm) to saturation (12–16 hours). It is recommended to start overnight culture for plasmid purification the next morning. The overnight culture should be transferred to 4° C if plasmid purification is planned to be performed later. It is recommended to use fresh culture to obtain high yield with less degradation of DNA.

- Use single isolated colony purified colony for inoculation.
- Use appropriate antibiotic.
- Do not use super rich media.
- Grow culture overnight for 12-16 hours.
- Use fresh culture for plasmid extraction.

Recipe

SOC	
Media for the initial propagation of cells after transformation	
Tryptone (casein peptone)	20.0 g/L
Yeast Extract	5.0 g/L
NaCl	0.50 g/L
$MgSO_4$	5.0 g/L
Add filter sterile glucose 3.6 g/L to autoclaved media	

Antibiotics

Antibiotic selection should be applied at all stages of growth. Many plasmids in use today do not contain the par locus, which ensures that the plasmids segregate equally during cell division in the absence of selective pressure. Daughter cells that do not receive plasmids will replicate much faster than plasmid-containing cells and can quickly take over the culture. The stability of the selective agent should also be taken into account. Resistance to ampicillin, for example, is mediated by β-lactamase which is encoded by the plasmid linked *bla* gene and which hydrolyzes ampicillin. Levels of ampicillin in the culture medium are thus continually depleted. This phenomenon is clearly demonstrated on ampicillin plates, where "satellite colonies" appear as the ampicillin is hydrolyzed in the vicinity of a growing colony. It is important to inoculate cultures from freshly prepared plates to ensure that the antibiotic is effective. Ampicillin is also very sensitive to temperature, and should be stored frozen in single-use aliquots.

Chloramphenicol amplification

The copy numbers of the current generation of plasmids are so high that selective amplification in the presence of chloramphenicol is not necessary to achieve high yields. However, when low copy number plasmids containing the pMB1 or ColE1 origin of replication are prepared, the yield can be improved by adding chloramphenicol (100 mg/liter) to amplify the copy number. Cultures of bacteria containing low-copy number plasmids amplified in the presence of chloramphenicol should be treated as if they contain high-copy-number plasmids when choosing the appropriate culture volumes.

Recipe

Ampicillin
Inhibits cell wall synthesis enzymes
Stock Solution 40 mg/ml in H_2O
Use at 80μg/ml (ie.2 μl of stock/ml medium)

Tetracycline
Binds to 30s ribosomal subunit. Inhibits ribosomal translocation
Stock Solution10 mg/ml in 50% Ethanol
Use at 50μg/ml (ie.5 μl of stock/ml medium) Note: Tetracycline HCl can be dissolved in water

Kanamycin
Binds to ribosomal components and inhibits protein synthesis
Stock Solution10 mg/ml in H_2O
Use at 50μg/ml (ie.5 μl of stock/ml medium)

Chloramphenicol
Binds to 50s ribosomal subunit and inhibits protein synthesis
Stock Solution 20 mg/ml in 50% EtOH
Use at 100μg/ml (ie.5 μl of stock/ml medium)

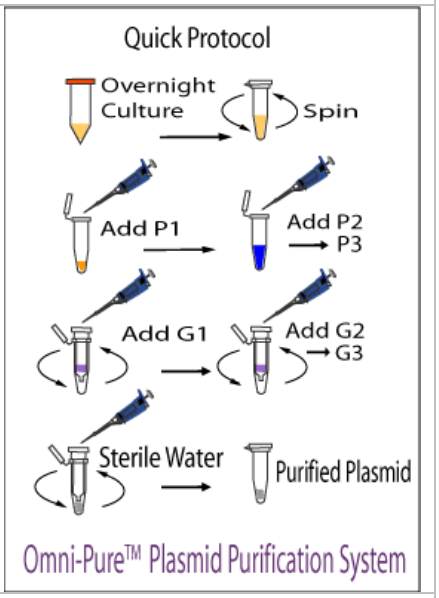
Plasmid Purification Protocol & Principle

Omni-Pure™ Plasmid DNA Purification Systems

Quick Protocol

20 Minute Mini-Prep Ultra Purification of Plasmid DNA

1. Spin down 2 X 1.5 ml of bacterial cells from an overnight culture.
2. Resuspend cells in 150 µl of P1.
3. Add 150 µl of P2. Mix gently.
4. Add 150 µl of P3 and place on ice.
5. Centrifuge at 12 K rpm for 1 minute.
6. Transfer supernatant to a fresh tube and add 450 µl of G1.
7. Transfer contents to a spin column, spin and discard eluent.
8. Add 400 µl of G2, spin and discard eluent.
9. Add 500 µl of G3, spin and discard eluent.
10. Elute with 50 µl sterile water or low salt buffer.



• Check P2 solution for SDS precipitation at low storage temperatures. Warming to 37° C will dissolve the SDS.
• Pre-chill P3 solution to 4° C

⚠ Solutions G1 and G2 contain guanidine hydrochloride, which can form highly reactive compounds when combined with bleach (sodium hypochlorite). If liquid containing this reagent is spilled, clean with water and, if necessary, an appropriate laboratory detergent. If the liquid contains potential pathogens, clean the affected area first with water and detergent, then disinfect with 1% sodium hypochlorite. Guanidine hydrochloride is also an irritant.
Solution G3 after reconstitution contains ethanol and is flammable.

Omni-Pure™ Plasmid DNA Purification System **Catalog No.: 40-4020-XX**

Detailed Protocol

20 Minute Mini-Prep Ultra Purification of Plasmid DNA

1. Spin down 1.5 ml of cultured bacterial cells for 1 minute at 3 K rpm in a microcentrifuge tube and remove the supernatant
2. Repeat step 1 once more to maximize bacterial cells.
3. Resuspend cells in 150 µl of Solution P1, mix gently.
4. Add 150 µl of Solution P2 and mix by inversion of tube. ***Do not mix by vortexing***. This can cause mechanical shearing of the bacterial chromosomal DNA, which will make it more difficult to remove in the next step.
5. Add 150 µl of Solution P3, mix gently by inversion and place on ice for 5 min.
6. Centrifuge for 1 min. at 12 K rpm.
7. Decant the supernatant into a fresh 1.5 ml microcentrifuge tube.
8. Add 450 µl of Solution G1 and mix thoroughly by vortexing.
9. Place a spin column in its collection tube and transfer the mixture obtained in step 8 to the spin column.
10. Centrifuge at 2 K rpm for 1 min. and discard the eluent
11. Add 400 µl of Solution G2.
12. Centrifuge at 2 K rpm for 1 min and discard the eluent.
13. Add 500 µl of Solution G3
14. Centrifuge at 2 K rpm for 1 min., and discard the eluent.
15. Centrifuge at 12 K rpm for 1 min. and discard eluent. This step is important to eliminate the last traces of solution G3, which contains ethanol. The presence of ethanol can interfere with the elution step.
16. Place the spin column in a fresh 1.5 ml microcentrifuge tube and add 50 µl of sterile deionized water and allow it to remain for 1 minute. Use hot 60° C water for quick efficient elution.
17. Centrifuge for 1 min. at maximum speed and collect the eluent. This contains ultra pure plasmid DNA.

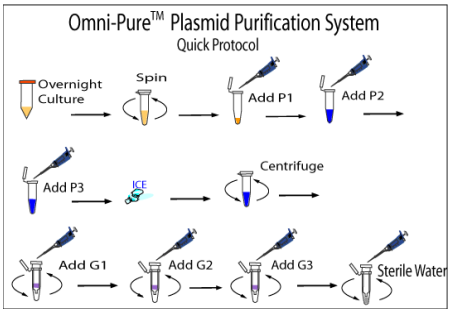
• Use filter barrier pipette tips to prevent cross contamination.

• Check Buffer P2 for SDS precipitation at low storage temperatures. Warming to 37° C will dissolve the SDS.
• Pre-chill Buffer P3 to 4° C
• Dilute G3 concentrate DNA wash solution prior to use.

Recipe

G3 DNA Wash Concentrate	
Dilution of G3 Concentrate With 100 % Ethanol Required Prior to Use	
G3 Concentrate	100% Ethanol To Add
15 ml	45 ml
75 ml	225 ml

Key Steps
• Ensure complete resuspension of cells in P1 solution.
• Do not vortex sample after adding P2 solution. Vortexing will shear chromosomal DNA into small pieces that may be indistinguishable from plasmid DNA and will be co-purified.



• Solutions G1 and G2 contain guanidine hydrochloride, which can form highly reactive compounds when combined with bleach (sodium hypochlorite). If liquid containing this reagent is spilled, clean with water and, if necessary, an appropriate laboratory detergent. If the liquid contains potential pathogens, clean the affected area first with water and detergent, then disinfect with 1% sodium hypochlorite. Guanidine hydrochloride is also an irritant.
• Solution G3 after reconstitution contains ethanol and is flammable.

Product Description

Introduction

The principle of plasmid extraction is established on the classical method of lysing the bacterial cells and selectively precipitating the large chromosomal DNA and proteins. The plasmid DNA remains in solution and is later recovered by ethanol precipitation or binding to silica particles followed by elution.

The Omni-Pure™ plasmid purification system is based on the above principle and is completely optimized by the formulation of chaotropic salt blends to yield ultra pure plasmid DNA in less than 30 minutes.

Plasmid DNA is efficiently released into the supernatant by modified bacterial lysis solution P2. In this solution proteins are completely denatured and bound with SDS on the surface. Potassium ion is an SDS precipitant under low pH condition. After neutralization with Buffer P3 containing high concentration of potassium ions, proteins bound with SDS are co-precipitated with the aggregate of SDS/potassium. Large un-sheared chromosomal DNA is trapped in the precipitated clump of proteins and is removed by centrifugation or filtration. The plasmid DNA remains in the supernatant and is further purified by adding chaotropic salts that completely denatures the DNA and is essentially being salted out.

The spin column method for plasmid DNA is based on the observation that in the presence of high concentrations of chaotropic salts, DNA will be absorbed tightly to glass particles. With spin column technology the glass is in the form of a thin membrane that is embedded into a small column that can be spun in a microcentrifuge. In the basic procedure, DNA is applied to the column under denaturing conditions in the presence of high concentrations of salt. After a brief spin in a microcentrifuge, the solution passes into a collection tube, leaving the DNA tightly bound to the glass membrane. Following a series of brief washes, the purified DNA can be eluted in a small volume of low salt buffer or sterile water. This procedure is applicable for the isolation of plasmid DNA from bacterial cells, removal of unwanted products from PCR reactions or enzyme digests, and especially for the extraction of DNA fragments from agarose gels. The spin column method is rapid, easy, and is ideally suited for handling multiple samples. The purified DNA is ready to use for any application, e.g., PCR, restriction enzyme digests, cloning, sequence analysis, labeling, etc. It should also be noted that an advantage of the spin column method over the glass bead procedure is that there is no possibility of carrying over particulate matter during the final elution step. This makes it the method of choice when the purified DNA is to be used for microinjection or capillary electrophoresis.

Plasmid DNA Binding to Spin Column Membrane

During the initial binding step, only DNA is absorbed to the glass membrane. Contaminants that may be present in the solution, such as primers, salts, proteins, unincorporated nucleotides, agarose, dyes, ethidium bromide, and detergents (e.g., SDS) do not bind to the glass membrane, and are efficiently removed during the washing steps with buffers G2 and G3. Any residual buffer G3, which may impede the quantitative elution of the DNA as well as interfere with subsequent enzymatic reactions, is removed by an additional high speed centrifugation step.

DNA Elution

In contrast to DNA absorption, DNA elution is enhanced by low salt concentrations and mild alkaline conditions. DNA can be eluted with either a low salt buffer (10 mM Tris-Cl, pH 8.0) or water. Elution is most efficient between pH 7.0 and 8.5. When using water to elute, make sure that the pH is within this range. Water that has been standing for a long period of time may have pH values as low as 5.5-6 due to absorption of CO_2. Usually, boiling the water 2-3 minutes in a microwave will remove dissolved CO_2 and brings it within an acceptable pH range. It should be noted, however, that DNA stored in pure water is more sensitive to degradation than when stored in buffered solutions. Therefore, it is recommended that DNA eluted with water be stored at -20° C. Elution with buffers containing EDTA should be avoided because it interferes with subsequent enzyme reactions.

For efficient elution with 50 µl, the elution buffer or water should be warmed to 60° C and allowed to remain in the column for 1 minute prior to centrifugation. However, elution can also be done with as little as 30 µl of elution buffer or water. When using less than 50 µl it is important to apply the elution buffer directly to the center of the glass membrane and allow it to sit for at least 2 minutes. To ensure quantitative elution it is recommended that following centrifugation, the eluent should be reapplied to the column, allowed to sit for 1 minute, and centrifuged a second time.

Improving Plasmid Yield

The Omni-Pure™ plasmid purification system typically yields ~5-10 µg plasmid DNA from 3ml culture of high copy number plasmid with an insert size of ~ 2 kb. Plasmids up to approximately 150 kb can be purified using the Omni-Pure™ plasmid purification system. Actual yields will depend on culture density, culture medium, plasmid copy number, insert size, and host strain. The protocol presented in this manual is for the isolation of plasmid DNA from E. coli.

- Plasmid size above 150 kb leads to lower yield of plasmid DNA.
- High copy number plasmids will have higher yield.
- High copy number plasmids with inserts larger than 40 kb will have lower yield.
- Do not grow bacterial culture longer than 14-16 hrs.
- Avoid E. coli strains, e.g. HB101 and JM100 series, with high carbohydrate content and high endonuclease activity.
- Host strains DH1, DH5α™ and XL-Blue yield high quality DNA.
- Do not incubate cells in cell lysis solution (P2) longer than a few minutes.
- Remove all traces of ethanol (after adding G3) before elution of plasmid DNA.

Plasmid Quality and Applications

The Omni-Pure™ plasmid purification system consistently yields ultra pure plasmid suitable for all molecular biology applications including the demanding purity required for fluorescent sequencing. The cause of poor quality plasmid DNA is usually associated with the use of host strains that have high carbohydrate and endonuclease content (e.g. HB101, JM 100 etc.). If you are using these strains for propagating transformants then it is recommended to improve the quality by ethanol precipitation or PEG precipitation.

Special consideration should be given to the concentration of DNA used for sequencing. Purified plasmid DNA must be within the proper concentration range for successful automated cycle sequencing (ideally 0.2µg/µl, not less than 0.1µg/µl). When working with plasmid DNA from low copy number plasmids, we strongly recommend that DNA concentrations be determined by agarose gel/ethidium bromide quantitation prior to any application. DNA quantitation by spectrophotometric methods is prone to errors at low concentrations and requires a large amount of sample for accurate determination.

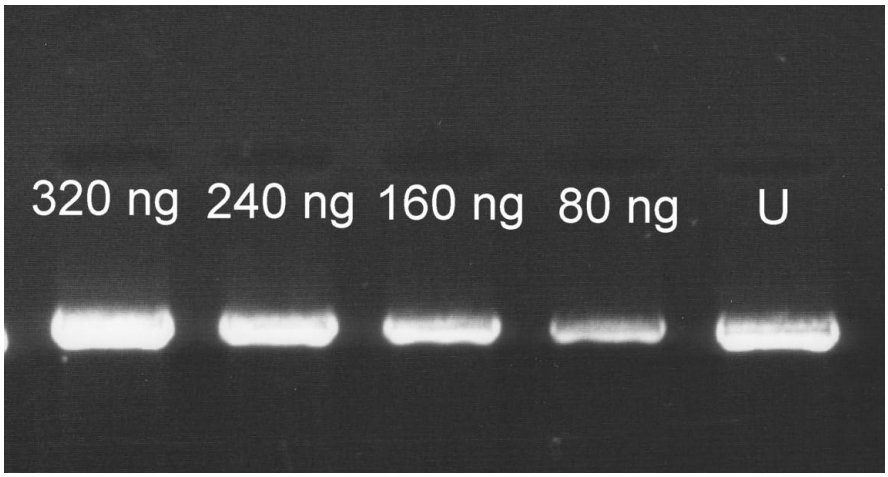
Spectrophotometric Determination of DNA Concentration

Measuring the optical density (OD) or absorbance at 260 nm (A_{260}) in a UV spectrophotometer is a relatively accurate method for calculating the concentration of DNA in an aqueous solution if a standard curve is meticulously prepared. An A_{260} of 1.0, using a 1 cm path length, corresponds to a DNA concentration of 50 µg/ml for double stranded DNA, 40 µg/ml for single stranded DNA and RNA and 33 µg/ml for single stranded oligonucleotides. However, this method is not suitable for determining concentrations of dilute solutions of DNA, as the sensitivity of this method is not very high. For reliable readings, the concentration of double stranded DNA must be greater than 1 µg/ml.

A simple, inexpensive method for the estimation of nanogram quantities of DNA is described in the following section. We recommend the use of agarose gel electrophoresis for routine approximate determination of DNA concentration.

Estimation of DNA Concentration by Agarose Gel Electrophoresis

The amount of DNA in a sample may be estimated by running the sample alongside standards containing known amounts of the same-sized DNA fragment. In the presence of ethidium bromide staining, the amount of sample DNA can be visually estimated by comparing the band intensity with that of the known standards.



An unknown amount of a 4 kb DNA fragment (unkown) was run alongside known quantities (indicated in nanograms) of the same DNA fragment. As estimated by visual comparison with the known standards, the unknown sample contained 240-320 ng of DNA.

Ethidium bromide is a carcinogen. Follow Health and Safety Procedures established by your institution.
Follow proper Hazardous Material Disposal procedures established by your institution.

• Use 0.1 µg of ethidium bromide solution for each ml of gel volume.

Agarose Gel Electrophoresis of DNA

Agarose gels are typically run at 20 to 150V. The upper voltage limit is the amount of heat produced. At room temperature about 5 Watts is correct for a minigel (Volts x Amps = Watts). At low voltages, migration is linearly proportional to voltage, but long DNA molecules migrate relatively faster in stronger fields. Migration is inversely proportional to the log of the fragment length; a log function also governs migration rate and gel concentration (0.5 to 2% for most purposes). Furthermore, supercoiled / circular DNA molecules migrate at different rates from linear molecules; single-stranded DNA and RNA migrate at similar rates, but usually faster than double-stranded DNA of the same length. Salt in the samples increases conductivity and, hence, migration rate.
The buffers used for most neutral agarose gels (the gel itself and the solution in which it lies) is 1 x TAE or 1 x TBE. Agarose powder is added to the buffer at room temperature, heated in a microwave and boiled slowly until the powder has dissolved. The gel is cast on a horizontal surface once the agarose has been cooled to 60° C (just cool enough to hold) and 0.1 µg of ethidium bromide solution is added for each ml of gel volume. At times, during removal of the comb, it is possible to tear the bottom of the sample wells, which results in sample leakage upon loading. This can be avoided by removing the comb after the gel has been placed in the running buffer.

• Use TAE buffer for most molecular biology agarose gel electrophoresis.

Recipe

1 X TAE Buffer
Agarose Gel Electrophoresis Buffer
40 mM Tris-Acetate pH 7.8
1 mM EDTA

1 X TBE
Agarose and Polyacrylamide Gel Electrophoresis Buffer
0.089 M Tris
0.089 M Boric Acid
0.002 M EDTA

Troubleshooting Guide

The suggestions in this section should be helpful in resolving the most common problems that may arise during DNA purification. In addition, the members of our Technical Support Services at Gene Link are always happy to answer any questions you may have regarding the procedures in this manual, as well as other applications pertinent to molecular biology.

Problem	Possible Cause	Suggestions
Low/ no recovery of DNA	Ethanol not added to the DNA Wash Solution	• Be sure that ethanol was added to the DNA wash buffer concentrate. If not, then repeat the entire procedure with correctly prepared wash buffer. • Be sure the pH of the water or buffer used for elution is between 7 and 8.5 • Repeated use of electrophoresis buffer can result in elevated pH values. If this is the case, replace with fresh buffer. • DNA will remain trapped in undissolved gel fragments. Make certain, by visual examination, that all of the gel has dissolved before adding glass beads or applying the solution to the spin column. The limit of the gel that can be handled by the spin column procedure is 400 mg. For gel slices larger than 400 mg, it is recommended that multiple spin columns be used.
	Overgrowth of bacterial culture by nontransformed bacteria	Make certain that antibiotics were used in all media, both liquid and solid. Do not culture bacteria longer than 24 hours. Optimal culture length is 12–16 hours.
	Bacterial culture too old	Inoculate antibiotic containing media with freshly isolated bacterial colony from an overnight plate.
Eluted DNA contains ssDNA which appears as a smear on an analytical gel	Cell lysis step was prolonged	• Repeat entire procedure with no longer than 3 minute in cell lysis buffer. • The ssDNA can be reannealed by heating the solution to 95° C for 2 minutes and allowing the tube to cool slowly to room temperature.
There appears to ethanol in the eluted DNA sample	Remaining traces of G3 that contains ethanol	• Following the final wash step, be sure to spin the column at maximum speed for 1 minute to remove the last traces of ethanol.
DNA yields on gel look low compared to spectrophotometer readings	Traces of contaminants may be present in the eluted DNA, which inflate the spectrophotometer readings	• Process DNA by adding 2 volumes of G1 and following the rest of the protocol onwards. • Phenol: chloroform extract and precipitate DNA, then wash with 70% ethanol before repeating spectrophotometer readings. Alternatively, quantitate the DNA by agarose gel/ethidium bromide electrophoresis for more accurate quantitation
Genomic DNA contamination	Vortexing or over mixing after addition of the Cell Lysis Solution	Do not vortex samples after addition of Cell Lysis Solution to prevent shearing of genomic DNA
No/ poor results with automated fluorescent sequencing	Too little DNA was added to the sequencing reaction. Plasmid concentration not accurately quantitated	Ethidium bromide gel electrophoresis must be used to accurately quantitate plasmid DNA.
	TE buffer was used for DNA elution	Ethanol precipitate and resuspend pellet in nuclease-free water. (The EDTA in TE buffer can interfere with downstream applications by chelating Mg^{2+}.)
DNA floats out of well during loading of agarose gel	Carryover of residual ethanol from Column Wash Solution	Follow directions for appropriate drying of resin by vacuum and centrifugation. If DNA has already been eluted, precipitate DNA and dry remaining ethanol from the DNA pellet prior to resuspension in nuclease-free water. Increase loading dye concentration to 2X.

References

1. Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615-619.

2. Cashion, P., Sathe, G., Javed, A. and Kuster, J. (1980) Nucl. Acids Res. 8, 1167-1185.

3. Cashion, P., Javed, A., Harrison, D., Seeley, J., Lentini, V. and Sathe, G. (1981) Hydrophobic chromatography of nucleic acids and proteins on tritylated agarose. In Gene amplification and analysis, Vol. 2. Analysis of nucleic acids by enzymatic methods. Ed. Chirikjian, J.G. and Papas, T.S. Elsevier North Holland, pp. 551-563.

4. K.A. Melzak et al. (1996) J. Colloid Interface Sci. (USA) 181, 635-644

5. Sambrook, J. et al., eds. (1989) Molecular cloning: a laboratory manual, 2nd ed., Cold Spring Harbor Laboratory Press

6. Birnboim, H.C. and Doly, J. (1979) A rapid alkaline lysis procedure for screening recombinant plasmid DNA. Nucl. Acids. Res. 7, 1513–1522.

7. Ausubel, F. M. et al., eds. (1991) Current protocols in molecular biology, Wiley Interscience, New York.

8. Birnboim, H.C. (1983) A rapid alkaline extraction method for the isolation of plasmid DNA. Methods Enzymol. 100, 243–255.

Ordering Information

Bacterial Culture Media		
Product	**Catalog No.**	**Unit Size**
Agar Type A Bacterial Culture Grade, 100 g	40-3301-10	100 g
Agar Type A Bacterial Culture Grade, 500 g	40-3301-05	100 g
Agar Type A Bacterial Culture Grade, 1 kg	40-3301-01	1 kg
Yeast Extract Bacterial Culture Grade, 100 g	40-4331-10	100 g
Yeast Extract Bacterial Culture Grade, 500 g	40-4331-10	500 g
Yeast Extract Bacterial Culture Grade, 1 k g	40-4331-10	1 kg
Casein Peptone (Type 1) Bacterial Culture Grade, 100 g	40-3305-10	100 g
Casein Peptone (Type 1) Bacterial Culture Grade, 500 g	40-3305-05	500 g
Casein Peptone (Type 1) Bacterial Culture Grade, 1 kg	40-3305-01	1 kg

Omni-Pure™ Plasmid DNA Purification Systems		
Product	**Catalog No.**	Unit Size*(Purifications)
Omni-Pure™ Plasmid DNA Purification System	40-4020-01	100
Omni-Pure™ Plasmid DNA Purification System	40-4020-05	500
*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.		

Related Products Ordering Information

Agarose Ultra Pure Molecular Biology Grade		
Product	**Catalog No.**	Unit Size
Agarose LE Molecular Biology Grade; 100 g	40-3010-10	100 g
Agarose LE Molecular Biology Grade; 500 g	40-3010-50	500 g
Agarose LE Molecular Biology Grade; 1 kg	40-3010-01	1 kg
Agarose HiRes Ultra Pure Molecular Biology Grade; 100 g	40-3015-10	100 g
Agarose HiRes Ultra Pure Molecular Biology Grade; 500 g	40-3015-50	500 g
Agarose HiRes Ultra Pure Molecular Biology Grade; 1 kg	40-3015-01	1 kg
Agarose Low Melt Ultra Pure Molecular Biology Grade; 100 g	40-3016-10	100 g
Agarose Low Melt Ultra Pure Molecular Biology Grade; 500 g	40-3016-50	500 g
Agarose Low Melt Ultra Pure Molecular Biology Grade; 1 kg	40-3016-01	1 kg

Product	Catalog No.	Unit Size
TAE Buffer; 50 X Concentrate	40-3007-01	100 mL
TAE Buffer; 50 X Concentrate	40-3007-05	500 mL
TBE Buffer; 5 X Concentrate	40-3008-10	1000 mL

Related Products Ordering Information

Buffers& Reagents

Product	Catalog No.	Unit Size
Hybwash A, Hybridization Wash Solution	40-5020-20	200 mL
Hybwash B, Hybridization Wash Solution	40-5021-10	100 mL
10x Washing buffer	40-5025-20	200 mL
10% Blocking solution	40-5026-10	100 mL
Seq. Loading buffer	40-5027-00	1 mL
10x AP Detection buffer	40-5031-10	100 mL
Lumisol™ I Hybridization Solution; contains formamide	40-5022-20	200 mL
Lumisol™ II Hybridization Solution; for non-toxic hybridizations	40-5023-20	200 mL
Lumisol™ III Hybridization Solution; for oligo probes	40-5024-20	200 mL

Omni-Marker™

Product	Catalog No.	Unit Size
Omni-Marker™ Universal unlabeled	40-3005-01	100 µL
Omni- Marker™ Universal unlabeled	40-3005-05	500 µL
Omni-Marker™ Universal unlabeled	40-3005-10	1 mL
Omni- Marker™ Low unlabeled	40-3006-01	100 µL
Omni-Marker™ Low unlabeled	40-3006-05	500 µL
Omni- Marker™ Low unlabeled	40-3006-10	1 mL
Omni-Marker™ GScan-2 Tamra labeled 50 bp - 600 bp	40-3062-01	100 µL
Omni-Marker™ GScan-2 Tamra labeled 50 bp - 600 bp	40-3062-05	500 µL

Loading Buffers

Product	Catalog No.	Unit Size
Loading Buffer 5X BPB/XC non-denaturing	40-3002-01	100 µL
Loading Buffer 5X BPB/XC non-denaturing	40-3002-10	1 mL
Loading Buffer 5X Orange G/XC non-denaturing	40-3004-01	100 µL
Loading Buffer 5X Orange G/XC non-denaturing	40-3004-10	1 mL
Loading Buffer 2X BPB/XC Denaturing for Sequencing	40-5027-01	100 µL
Loading Buffer 2X BPB/XC Denaturing for Sequencing	40-5027-10	1 mL

All Gene Link products are for research use only.

Related Products Ordering Information

Omni-Pure™ Plasmid DNA Purification Systems		
Product	**Catalog No.**	Unit Size*(Purifications)
Omni-Pure™ Plasmid DNA Purification System	40-4020-01	100
Omni-Pure™ Plasmid DNA Purification System	40-4020-05	500
*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.		

Omni-Clean™ Gel DNA Purification and Concentration Systems		
Product	**Catalog No.**	Unit Size*(Purifications)
Omni-Clean™ Gel DNA Beads Purification System	40-4110-10	100
Omni-Clean™ Gel DNA Beads Purification System	40-4110-50	500
Omni-Clean™ Gel DNA Spin Column Purification System	40-4120-10	100
Omni-Clean™ Gel DNA Spin Column Purification System	40-4120-50	500
Omni-Clean™ DNA Beads Concentration System	40-4130-10	100
Omni-Clean™ DNA Beads Concentration System	40-4130-50	500
Omni-Clean™ DNA Spin Column Concentration System	40-4140-10	100
Omni-Clean™ DNA Spin Column Concentration System	40-4140-50	500
*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.		

Omni-Pure™ DNA & RNA Purification Systems		
Product	**Catalog No.**	Unit Size*(Purifications)
Omni-Pure™ Blood DNA Purification System	40-4010-01	100
Omni-Pure™ Blood DNA Purification System	40-4010-05	500
Omni-Pure™ Blood DNA Purification System	40-4010-10	1000
Omni-Pure™ Tissue DNA Purification System	40-4050-01	100
Omni-Pure™ Tissue DNA Purification System	40-4050-05	500
Omni-Pure™ Tissue DNA Purification System	40-4050-10	1000
Omni-Pure™ Plant DNA Purification System	40-4060-01	100
Omni-Pure™ Plant DNA Purification System	40-4060-05	500
Omni-Pure™ Plant DNA Purification System	40-4060-10	1000
Omni-Pure™ Viral DNA Purification System	40-3720-01	100
Omni-Pure™ Viral DNA Purification System	40-3720-05	500
Omni-Pure™ Microbial DNA Purification System	40-3700-01	100
Omni-Pure™ Microbial DNA Purification System	40-3700-05	500
Omni-Pure™ Viral RNA Purification System	40-3650-01	100
Omni-Pure™ Viral RNA Purification System	40-3650-05	500
*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.		

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Notes: