Genetic Tools & Reagents

Product Manual

Omni-Pure™ Mini-Prep Plasmid DNA Purification Systems

Catalog No: 40-4020-XX

Gene Link™

Contents

Gene Link™

Material Supplied

Omni-Pure™ Plasmid DNA Purification Systems; Mini-Prep			
	Product	**Catalog No.**	**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.

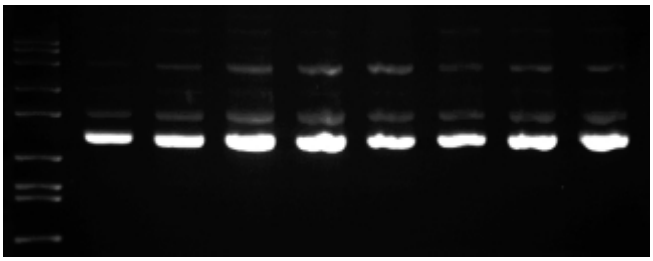
Omni-Pure™ Plasmid DNA Purification Systems; Mini-Prep				
Product	**Catalog No.**	**Size**	**Catalog No.**	**Size**
Omni-Pure™ Plasmid DNA Purification System	40-4020-01	100	40-4020-05	500
	❑		❑	
Materials Supplied				
P1-Cell Suspension Solution	40-4021-02	20 ml	40-4021-80	80 ml
P2-Cell Lysis Solution	40-4022-02	20 ml	40-4022-80	80 ml
P3-Chromosomal DNA Precipitation Solution	40-4023-02	20 ml	40-4023-80	80 ml
G1-Chaotrope Blend Solution I	40-4024-05	50 ml	40-4024-25	250 ml
G2- Chaotrope Blend Solution II	40-4025-05	50 ml	40-4025-25	250 ml
*G3-DNA Wash solution; 2x concentrate supplied	40-4026-03	30 ml	40-4026-15	150 ml
Spin Column	40-4121-01	100	40-4121-01	100 x 5

*G3- DNA Wash Solution 2x Concentrate Dilution			
Dilution Required Prior To Use			
Product	**Catalog No.**	**Size**	**Volume of Absolute Ethanol To Add**
*G3-DNA Wash solution; 2x concentrate supplied	40-4026-03	30 ml	30 ml
*G3-DNA Wash solution; 2x concentrate supplied	40-4026-15	150 ml	150 ml

Omni-Pure™ Plasmid DNA Purification System

Routine mini-preps of plasmid extraction are made even easier with consistent performance. Purification can be performed with a maximum of 3 ml of cells yielding up to 20 µg of purified DNA. The convenient spin column method can be scaled up by using multiple columns and processed in less than 30 minutes. The purified DNA is of high quality suitable for all molecular biology applications including direct use in fluorescent automated sequencing methods.

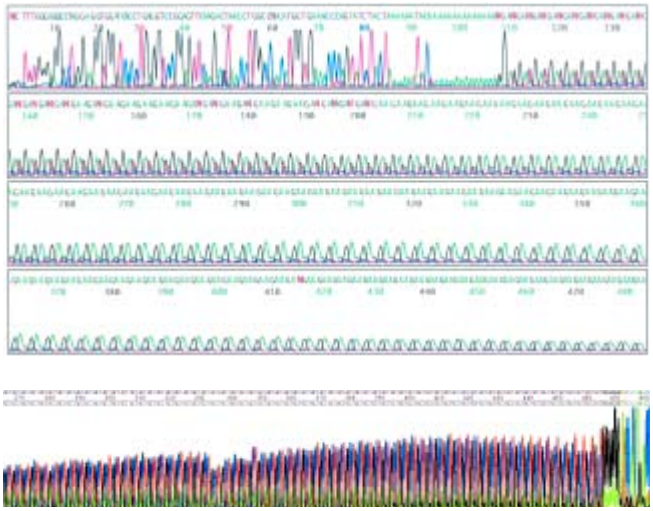
Facile and rapid purification of DNA from varied sources can be performed using the Omni-Pure™ series of DNA, RNA and plasmid purification systems. Each purification system has been formulated, optimized and designed to yield the highest purity available with the starting sample volume specially geared towards the desired downstream application.



Replicates of plasmid purification using the Omni-Pure™ Plasmid DNA Purification System.

Omni-Pure™ System

- Ultra Purified High Yield Plasmid DNA
- No Toxic Reagents
- Rapid Purification Protocols
- Suitable for All Molecular Biology Applications
- Convenient Optimized Reagents
- Easy Spin Column Format
- Genomic DNA Purification
- Viral DNA & RNA Purification
- Microbial DNA Purification



Friedreich's Ataxia Control DNA with 110 GAA repeats (top) and Myotonic Dystrophy Control DNA with 129 CTG repeats (bottom) sequencing electropherograms. Sequencing these triple repeats requires ultra clean DNA. Plasmids were purified using the Omni-Pure™ system and processed for automated sequencing.

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. The potassium ion is a SDS precipitant under low pH conditions. After neutralization with Buffer P3 containing a 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 denature the DNA. It 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 binds 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 the buffers. Any residual G3 buffer, 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 bring 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 EDTA 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 3 minutes 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 3 minutes. To ensure quantitative elution it is recommended that following centrifugation, the eluent should be reapplied to the column, allowed to sit for 3 minutes, and centrifuged a second time.

Improving Plasmid Yield

The Omni-Pure™ Plasmid Purification System typically yields ~5-10 µg of plasmid DNA from a 3 ml culture of a 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 DNA 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 0.2 µg/µl, not less than 0.1 µg/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 the sample for accurate determination.

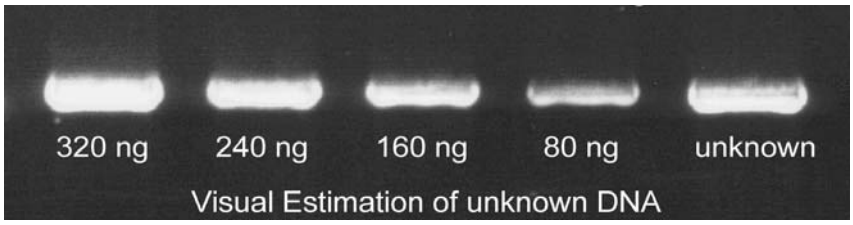
Spectrophotometric Determination of DNA Concentration

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 along side of 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 (unknown) was run along side of 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) are 1x TAE or 1x 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

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

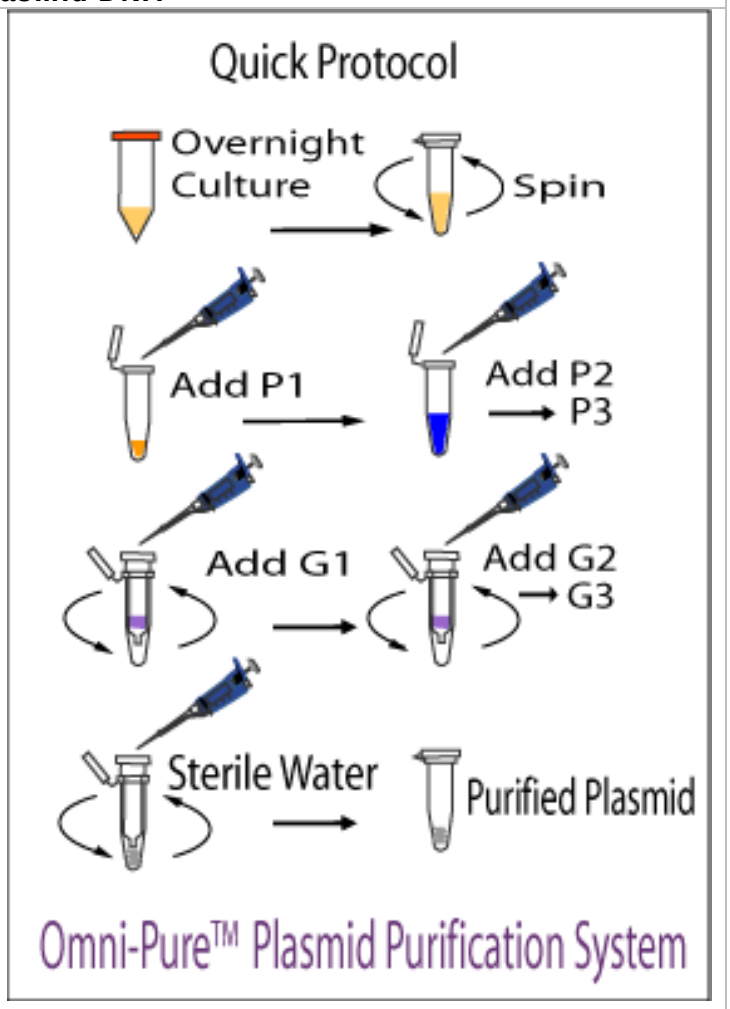
1x TBE
Agarose and Polyacrylamide Gel Electrophoresis Buffer
0.089 M Tris
0.089 M Boric Acid
0.002 M EDTA

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 12K 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.

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 5K 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 and keep for 5 minutes.
4. Add 150 µl of Solution P2 and mix by inversion of tube and keep for 5 minutes. ***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 minutes.
6. Centrifuge for 1 minute at 12K 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 4K rpm for 1 min and discard the eluent.
11. Add 400 µl of Solution G2.
12. Centrifuge at 4K rpm for 1 min and discard the eluent.
13. Add 500 µl of Solution G3.
14. Centrifuge at 4K rpm for 1 min and discard the eluent.
15. Centrifuge at 12K rpm for 1 minute 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.
1. 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 3 minutes. Use hot 60°C water for quick efficient elution.
16. Centrifuge for 1 minute 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 2x Concentrate	100% Ethanol To Add
30 ml	30 ml
150 ml	150 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.

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.
	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 minutes in the cell lysis buffer. • The ssDNA can be re-annealed 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 contain 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.

Appendix

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 plasmids.

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 8 ml/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 cells. 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 carbohydrates and endonuclease activity.
- 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 an overnight culture for plasmid purification the next morning. The overnight culture should be transferred to 4°C if it is planned for plasmid purification to be performed later. It is recommended to use fresh culture to obtain high yield with less degradation of DNA.

- Use single isolated 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 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.

Recipe

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

Tetracycline
Binds to 30s ribosomal subunit and inhibits ribosomal translocation
Stock Solution 10 mg/ml in 50% Ethanol
Use at 50 μg/ml (i.e., 5 μl of stock/ml medium) Note: Tetracycline HCl can be dissolved in water

Kanamycin
Binds to ribosomal components and inhibits protein synthesis
Stock Solution 10 mg/ml in H_2O
Use at 50 μg/ml (i.e., 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 100 μg/ml (i.e., 5 μl of stock/ml medium)

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.

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PCR Components and Analysis
PCR buffer conditions vary and it is imperative to optimize buffer conditions for each amplification reaction. At Gene Link most amplification reactions have been optimized to work with the following standard buffer condition, unless otherwise indicated.

Recipe

Standard Gene Link PCR Buffer Composition	
10x PCR buffer	**1x PCR buffer**
100 mM Tris-HCl pH 8.3	10 mM
500 mM KCl	50 mM
15 mM $MgCl_2$	1.5 mM
0.01% Gelatin	0.001%

dNTP Concentration
Standard dNTP concentration of 0.2 mM of each base is used. See section on PCR additives when dNTP concentration is changed.

Recipe

2.0 mM dNTP Stock Solution Preparation*	
Component	**Volume**
100 mM dGTP	100 µl
100 mM dATP	100 µl
100 mM dTTP	100 µl
100 mM dCTP	100 µl
Water	4.6 ml
Total Volume	5 ml
*Aliquot and freeze	

$MgCl_2$ Concentration
The concentration of Mg^{2+} will vary from 1-5 mM, depending upon primers and substrate. Since Mg^{2+} ions form complexes with dNTPs, primers and DNA templates, the optimal concentration of $MgCl_2$ has to be selected for each experiment. Low Mg^{2+} ion concentration results in a low yield of PCR product, and high concentrations increase the yield of non-specific products and promote mis-incorporation. Lower Mg^{2+} concentrations are desirable when fidelity of DNA synthesis is critical. The recommended range of $MgCl_2$ concentration is 1-4 mM, under the standard reaction conditions specified. At Gene Link, using the standard PCR buffer with KCl, a final dNTP concentration of 0.2 mM, a $MgCl_2$ concentration of 1.5 mM is used in most cases. If the DNA samples contain EDTA or other chelators, the $MgCl_2$ concentration in the reaction mixture should be raised proportionally. Given below is a $MgCl_2$ concentration calculation and addition table using a stock solution of 25 mM $MgCl_2$.

$MgCl_2$ Concentration & Addition Table								
Final concentration of $MgCl_2$ in 50 µl reaction mix, (mM)	1.0	1.25	1.5	1.75	2.0	2.5	3.0	4.0
Volume of 25 mM $MgCl_2$, (µl)	2	2.5	3	3.5	4	5	6	8

Primer Concentration
The final concentration of primers in a PCR reaction is usually 0.5 to 1 µM (micromolar). This is equivalent to 0.5 to 1 pmol/µl. For a 100 µl reaction, add 50 to 100 pmols. At Gene Link we use 0.5 pmol/µl in the final reaction.

Always use filter barrier pipette tips to prevent cross contamination

Genemer™ Reconstitution
Stock Primer Mix: Dissolve the supplied 10 nmols of lyophilized Genemer™ in 100 µl sterile TE. The 10 nmols of primers when dissolved in 100 µl will give a solution of 100 µM, i.e., 100 pmols/µl.

Primer Mix: Prepare a 10 pmols/µl Primer Mix solution by a ten fold dilution of the stock primer mix.

Example: Add 180 µl sterile TE to a new tube, to this tube add 20 µl of primer stock solution. Label this tube as Primer Mix 10 pmols/µl.

Recipe

TE Buffer pH 7.5 Composition
1x TE Buffer pH 7.5
10 mM Tris-HCl pH 7.5
1 mM EDTA

Amplification Thermal Cycling

Hot Start: It is essential to have a 'Hot Start' profile for amplification of any fragment from a complex template like human genomic DNA. Taq polymerase has low activity at room temperature and it is essential to minimize any mis-priming in the first cycle of amplification. A typical hot start profile is given below. Various enzyme preparations are available which are activated by heat in the first cycle. A simple hot start protocol is given below that can be used with regular Taq polymerase. See the section on PCR additives for amplification of products from high GC content templates.

Hot Start		
Step	**Time & Temperature**	**Cycles**
Initial Denaturation	95 °C for 5 minutes	1
Annealing	60 °C Hold Infinity	Hold
Comments: Add Taq premix while on hold.		

Amplification File

The initial denaturation step at 94°C for 30 seconds is sufficient for all templates. The number of cycles is usually set to 30 and is sufficient to amplify 1-10 µg of product depending on the initial concentration of template. A higher number of cycles from 35-45 cycles may be used, but internal priming on the product and over amplification of unwanted bands often result from over-cycling. Generally, it is better to focus on optimizing reaction conditions than to go beyond 35 cycles.

Typical Amplification File			
Step	**Temperature**	**Time**	**Cycles**
Denaturation	94°C	30 sec.	30
Annealing	*	30 sec.	
Elongation	72°C	30 sec.	
Fill in Extension	72°C	7 minutes	1
Hold	4°C	Infinity	Hold
*Based on the T_m of the primers. Usually varies from 50°C to 65°C			

PCR Premix Preparation (PP)		
Component	**1x 50 µl Rxn.**	**10x 50 µl Rxns.**
Sterile Water	32 µl	320 µl
10x PCR Buffer	4.5 µl	45 µl
2.0 mM dNTP	5 µl	50 µl
10 pmol/µl Primer Mix	2.5 µl	25 µl
Taq Enzyme Mix (EM) See below for preparation	5 µl	50 µl
Template DNA (~500 ng)	1-2 µl	Add 1-2 µl DNA to each tube
Total Volume	50 µl	
Keep on ice during set up. After adding template start PCR File		

•Program your thermal cycler instrument with an amplification profile prior to beginning the amplification protocol. Consult your appropriate instrument manufacturer's manual.

Recipe

Typical PCR Premix (/50µl)	
Component	**Volume**
10x PCR Buffer	5 µl
2.0 mM dNTP mix (each)	5 µl
Primer Mix (10 pmol/µl each) or 2.5 µl of 10 pmol/µl of individual primer (final 25 pmol of each primer/50 µl)	2.5 µl
H_2O	37.5 µl
Total Volume	50 µl

Recipe

PCR reaction (/50µl)	
Component	**Volume**
PCR premix	45 µl
100 ng/µl diluted DNA	1 µl
Hot start and then add	
Taq premix	5 µl

Recipe

Taq Premix (/50µl)	
Component	**Volume**
PCR Premix	6 µl
Taq polymerase (5 units/µl)	0.25µl
Add 5 µl/50 µl rxn after initial denaturation	
Use 2.5 units of Taq for 100 µl reactions. Taq is usually supplied at a concentration of 5 units/µl	

•The PCR premix preparation protocol is written considering that more than one amplification reaction will be performed at the same time. If only one reaction is planned then there is no need to prepare the Taq Enzyme Mix (EM).

Recipe

Gene Link PCR Buffer
1x PCR Buffer
10 mM Tris-HCl pH 8.3
50 mM KCl
1.5 mM $MgCl_2$
0.001% Gelatin

PCR Additives

DNA polymerases need to elongate rapidly and accurately to function effectively *in vivo* and *in vitro*, yet certain DNA regions appear to interfere with their progress. One common problem are pause sites, at which DNA polymerase molecules cease elongation for varying lengths of time. Many strong DNA polymerase pauses are at the beginnings of regions of strong secondary structure such as template hairpins (1). Taq polymerase used in PCR suffers the same fate and GC-rich DNA sequences often require laborious work to optimize the amplification assay. The GC-rich sequences possess high thermal and structural stability, presumably because the high duplex melting temperature that permits stable secondary structures to form, thus preventing completion of a faithful replication (2).

Nucleotide analog 7-deaza dGTP is effective in reducing the secondary structure associated with the GC-rich region by reducing the duplex stability (4). Betaine, DMSO and formamide reduce the T_m and the complex secondary structure, thus the duplex stability (1-5). Tetramethyl ammonium chloride (TMAC) actually increases the specificity of hybridization and increases the T_m. The use of TMAC is recommended in PCR conditions using degenerate primers.

These PCR additives and enhancing agents have been used to increase the yield, specificity and consistency of PCR reactions. These additives may have beneficial effects on some amplifications and it is impossible to predict which agents will be useful in a particular context and therefore they must be empirically tested for each combination of template and primers.

PCR Additives		
Additive	**Purpose & Function**	**Concentration**
7-deaza-2'-deoxyguanosine; 7-deaza dGTP	GC-rich region amplification. Reduces the stability of duplex DNA	Totally replace dGTP with 7-deaza dGTP; or use 7-deaza dGTP: dGTP at 3:1
Betaine (N,N,N-trimethylglycine = [carboxymethyl] trimethyl ammonium)	Reduces T_m facilitating GC-rich region amplification. Reduces duplex stability	Use 3.5 M to 0.1 M Betaine. Be sure to use Betaine or Betaine (mono)hydrate and **not** Betaine HCl.
BSA (bovine serum albumin)	BSA has proven particularly useful when attempting to amplify ancient DNA or templates which contain PCR inhibitors such as melanin.	BSA concentration of 0.01 µg/µl to 0.1 µg/ µl can be used.
DMSO (dimethyl sulfoxide)	DMSO is thought to reduce secondary structure and is particularly useful for GC-rich templates.	DMSO at 2-10% may be necessary for amplification of some templates, however 10% DMSO can reduce *Taq* polymerase activity by up to 50% so it should not be used routinely.
Formamide	Reduces secondary structure and is particularly useful for GC-rich templates.	Formamide is generally used at 1-5%. Do not exceed 10%.
Non-ionic detergents, e.g., Triton X-100, Tween 20 or Nonidet P-40 (NP-40)	Non-ionic detergents stabilize *Taq* polymerase and may also supress the formation of secondary structure.	0.1-1% Triton X-100, Tween 20 or NP-40 may increase yield but may also increase non-specific amplification. As little as 0.01% SDS contamination of the template DNA (left-over from the extraction procedure) can inhibit PCR by reducing *Taq* polymerase activity to as low as 10%, however, inclusion of 0.5% Tween-20 or -40 will effectively neutralize this effect.
TMAC (tetramethyl ammonium chloride)	TMAC is used to reduce potential DNA-RNA mismatch and improve the stringency of hybridization reactions. It increases T_m and minimizes mis-pairing.	TMAC is generally used at a final concentration of 15-100 mM to eliminate non-specific priming.

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Ordering Information

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Omni-Pure™ Plasmid DNA Purification System	40-4020-01	100
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*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

Related Products Ordering Information

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*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

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