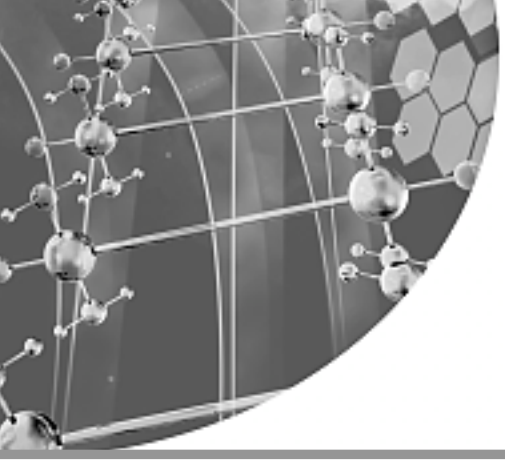

Manual Phase Lock Gel™ (PLG)

For convenient phenol or
phenol/chloroform extractions of
nucleic acids

Contents

Introduction

Phase Lock Gel™ (PLG) provides increased protection and ease of handling when working with standard organic extraction mixtures[14]. PLG acts as a barrier between the organic and aqueous phases, allowing the nucleic-acid-containing phase to be easily decanted or pipetted off - saving time while optimizing the recovery of nucleic acids. The use of PLG can result in the recovery of 20 to 30% more nucleic acid than with traditional methods. Multiple extractions can be performed in the same tube as long as maximum sample volume is not exceeded.

After organic extraction, it is often difficult to recover nucleic acid in the aqueous, upper phase that is free from the denatured protein present at the aqueous and organic phase interface. Phase Lock Gel (PLG) present during Phenol, Phenol:Chloroform, and Chloroform:Isoamyl Alcohol extractions migrates under centrifugal force to form a seal between the organic and aqueous phases. The organic phase and the interface material are effectively trapped below the PLG. The barrier is sufficiently durable that the aqueous upper phase, containing the nucleic acid, can then be recovered quantitatively by simply decanting or pipetting to a fresh tube.

PLG is inert, heat stable, and does not interfere with standard nucleic acid restriction and modification enzymes. In fact, many of the reactions can be carried out in the presence of PLG at the appropriate temperature and then terminated by extraction with Phenol or Phenol:Chloroform. PLG can be present during the heat inactivation of enzymes (65°C for 10 minutes) prior to the organic extraction.

The ability of PLG to separate the phases is based on the density differences of the aqueous and organic media. The organic layer must have a higher density than the PLG and aqueous phase, and the PLG must have a higher density than the aqueous phase. High salt and protein concentrations in the aqueous phase have an effect, after mixing with the organic, on both the aqueous and organic phase densities. Different organic phase formulations also vary in density. When choosing a type of PLG, the composition of all components must be taken into consideration. For optimum phase separation, the compositions of the aqueous phase, organic phase, and PLG must be compatible. As a result, PLG is offered in two different density formulations: Light (L) and Heavy (H). Please consult the table in section 6 for the formulation that fits your application.

Precautions and warnings

When using PLG, keep in mind that Phenol and Chloroform are hazardous chemicals. Appropriate safety apparel such as lab coat, gloves, and eye protection should be worn. For more information, please consult the appropriate material safety data sheets, which are available for this kit online at www.5 PRIME.com/msds.

Materials

Materials supplied with the kits

Various tube sizes are available with pre-dispensed PLG Light or Heavy. Sample volume ranges are indicated for each tube size.

PLG tube size	Sample volume	Tube color
0.5 ml, Heavy	10-150 µl	yellow
0.5 ml, Light	10-150 µl	green
1.5 ml, Heavy	100-500 µl	yellow
1.5 ml, Light	100-500 µl	green
2 ml, Heavy	10-750 µl	yellow
2 ml, Light	10-750 µl	green
15 ml, Heavy and Light	1-6 ml	† clear
50 ml, Heavy and Light	5-20 ml	† clear

† PLG Heavy is opaque while PLG Light is translucent.

→ Maximal centrifugal force for 15 ml and 50 ml conical tubes is 3500 x g.

Storage and stability

Store Phase Lock Gel at room temperature. Do not freeze.

Phase Lock Gel is stable for at least one year when stored as described.

Quality assurance

Phase Lock Gel Heavy is functionally tested for RNA extraction and phasing. The aqueous phase must be clear and the organic phase must be entirely below the gel. RNA is tested for quantity and quality by spectrophotometric assay and formaldehyde agarose gel electrophoresis.

Phase Lock Gel Light is functionally tested for phasing with NaCl and H_2O saturated phenol or BSA and phenol. The PLG barrier must be uniform and intact between the water and organic phases. The organic phase must be entirely below the gel.

Applications and compatibility

Organic phase compatibility

Aqueous phase	Organic Phase			
	PCL	CI	H_2O or Tris-buffer saturated PC	H_2O or Tris-buffer saturated phenol
< 0.5 M NaCl[a]	L, H	L, H	L, H	L
< 1 mg/ml BSA[a]	L, H	L, H	L, H	L
Plasmid DNA isolation[b]	H	H	H	O
Genomic DNA isolation[c]	L, H	L, H	L, H	L
RNA isolation[d]	H	H	H	O

L = Light H = Heavy
O = This combination of aqueous and organic phases is not suitable for use with Phase Lock Gel.
PCI = Phenol:Chloroform:Isoamyl Alcohol (25:24:1)
CI = Chloroform:Isoamyl Alcohol (24:1) PC = Phenol:Chloroform (1:1)

a. For optimum results with PLG Light, the starting sample should not exceed 0.5 M NaCl or 1 mg/ml protein. Samples exceeding these concentrations should be diluted prior to extraction. If dilution is inappropriate, extractions may be performed with PLG Heavy in combination with PCI, PC, or CI. PLG Heavy is not compatible with water- or Tris-buffer saturated Phenol (pH 8.0) as shown in the table.

b. Bacterial cleared lysates prepared according to alkaline lysis procedure.[7]

c. Protocols in which nuclei are first pelleted from cells lysed in the presence of 1% Triton X-100, and then lysed in saline/EDTA and SDS with or without the addition of Proteinase K.[11]

d. Preparation of total RNA using Guanidinium Isothiocyanate in conjunction with organic extraction.[5,6] PLG is not recommended for use with RNAzol®. PLG Heavy can be used in combination with TRIzol® or RNA-Isol Lysis Reagent for Total RNA isolation in most cases. Use the volumes recommended in the TRIzol® or RNA-Isol Lysis Reagent protocol and the centrifuge speeds and times in the PLG protocol. In some special applications, it may be necessary to add up to another volume of chloroform to achieve optimal phase separation.

PLG Light and Heavy facilitate most applications that require extraction with organic solvents.[14]

PLG Light can be used to improve the recovery of DNA fragments from Low Melting Point (LMP) Agarose with only minor changes to the standard protocol (see page 9). It may be used in the standard protocols for the preparation of plasmid DNA from *E. coli* (see page 16), phagemid DNA from M13-type phage (see page 10), and phage DNA from lambda (see page 12). PLG Light also may be used for isolating high molecular weight genomic DNA from blood, cultured cells and tissue (see page 13).

PLG Heavy may be used to prepare plasmid DNA from *E. coli* (see page 16) for the preparation of total RNA by homogenization in Guanidinium Isothiocyanate followed by organic extraction (see page 18), and for isolating genomic DNA from mouse tail (see page 15).

Protocol

If not otherwise stated, all centrifugation is at room temperature, and expressed at r_{max}.

→ Caution: Be certain to use the correct centrifuge tube adapters so that tubes are properly supported on the sides and bottom.

Tube size	Sample volume
0.5 ml	10 - 150 µl
1.5 ml	100 - 500 µl
2 ml	100 - 750 µl
15 ml	1 - 6 ml
50 ml	5 - 20 ml

PLG 0.5 ml, 1.5 ml and 2 ml Heavy and Light

1. Immediately prior to use, pellet Phase Lock Gel (PLG) at 12,000 - 16,000 x g in a microcentrifuge for 20 to 30 seconds.
2. Add 10 to 150 µl (PLG 0.5 ml), 100 to 500 µl (PLG 1.5 ml) or 100 to 750 µl (PLG 2 ml) of aqueous sample and an equal volume of organic extraction solvent directly to the pre-spun PLG tube.
3. Thoroughly mix the organic and aqueous phases to form a transiently homogeneous suspension. Do not vortex.

4. Centrifuge at 12,000 - 16,000 x g for 5 minutes to separate the phases. The PLG will form a barrier between the aqueous and organic phases. A small amount of PLG may remain in the bottom of the tube. If a second extraction is necessary, and maximum tube volume is not exceeded, more organic extraction solvent can be added to the same tube, mixed, and re-centrifuged.
5. Carefully decant or pipet off nucleic-acid-containing aqueous upper phase to a fresh tube.
6. Precipitate the nucleic acid by adding salt, alcohol, and carrier (if needed) as per your protocols and applications.

PLG 15 ml and 50 ml Heavy and Light

1. Immediately prior to use, pellet Phase Lock Gel (PLG) by centrifugation at 1500 x g for1 to 2 minutes.
2. Add 1 to 6 ml (PLG 15 ml) or 5 to 20 ml (PLG 50 ml) of aqueous sample and an equal volume of organic extraction solvent directly to the pre-spun PLG tube.
3. Thoroughly mix the organic and aqueous phases to form a transiently homogeneous suspension. Do not vortex.
4. Centrifuge for 5 minutes at 1500 x g to separate the phases. The PLG will form a barrier between the aqueous and organic phases. A small amount of PLG may remain in the bottom of the tube. If a second extraction is necessary, and the maximum tube volume is not exceeded, more organic extraction solvent can be added to the same tube, mixed, and re-centrifuged.
5. Carefully decant or pipet off nucleic-acid-containing aqueous upper phase to a fresh tube.
6. Precipitate the nucleic acid by adding salt, alcohol, and carrier (if needed) as per your protocols and applications.

Applications

Recovery of DNA from LMP agarose[1]

1. Resolve DNA fragments on a Low Melting Point (LMP) agarose gel in 1x Tris-Acetate- EDTA (TAE) buffer. Tris-Borate-EDTA (TBE) is not recommended as TBE gels are much more difficult to solubilize.
2. Stain gel with Ethidium Bromide, visualize with a longwave UV light, and carefully cut out the band(s) of interest with a sharp razor blade.
   → Caution: Wear gloves when handling Ethidium Bromide stained gels.
   → Caution: Wear safety glasses or a face shield when using a UV light.
3. Transfer slice to a pre-spun (12,000 x g for 20 to 30 seconds), pre-weighed PLG 2 ml Light tube and determine the weight of the slice.

4. Add a volume (in µl) of TE (10 mM Tris-Cl, 1 mM EDTA, pH 8.0) equivalent to 5x the weight (in mg) of the slice and melt the LMP agarose in the TE in an thermomixer set at 65°C for 5 to 10 minutes. If a thermomixer is not available a heat block can be used with intermittent mixing.
5. Mix well to ensure LMP agarose slice is fully dissolved, allow the dissolved sample to come to room temperature, and then add an equal volume of room temperature Tris- buffer saturated Phenol (pH 8.0) to the sample and mix until homogeneous.
   → Do not vortex.
6. Centrifuge at full speed (12,000 x g or greater in a microcentrifuge) for 2 minutes to separate the phases.
   → If the resulting aqueous phase still appears cloudy, the extraction should be repeated with room temperature Tris-buffer saturated Phenol (pH 8.0).
7. Recover aqueous phase to a fresh PLG 2 ml Light tube and extract with an equivalent volume of room temperature Phenol:Chloroform:Isoamyl Alcohol (PCI, 25:24:1).
   → Do not vortex.
8. Centrifuge as in step 6 above. Recover aqueous phase to a fresh PLG 2 ml Light tube, and extract with an equivalent volume of room temperature Chloroform:Isoamyl Alcohol (CI, 24:1).
9. Centrifuge as in step 6 above and recover the aqueous phase to a suitably sized microcentrifuge tube.
10. Add 0.25x volume of 10 M Ammonium Acetate and 2.5x volume of 100% Ethanol to the sample and mix well.
11. Incubate at room temperature for 20 minutes, pellet by centrifugation, wash pellet two to three times with cold 70% Ethanol, air-dry pellet, and resuspend in a suitable buffer.

M13/Phagemid DNA extraction protocol

Efficiency of the standard protocols[9,10] for production of single-stranded DNA may be improved through the use of PLG.[3]

1. Propagate M13 phage by one of the standard procedures:
   → For producing M13 ssDNA, mix 2.5 ml plating bacteria (5 to 7 hours, 50 ml culture; stored at 4°C for no more than 3 days) with 300 µl M13 phage stock (1 x 10^{11} pfu/ml) and incubate 5 minutes at room temperature to allow phage to interact with the bacterial phage receptors. The phage-bacteria culture is added to 250 ml LB broth and incubated for 5 to 7 hours at 37°C with sufficient agitation for good aeration of the culture.

→ For producing phagemid ssDNA, suspend a colony of phagemid transformed bacteria in a 15 ml culture tube containing 3 ml LB broth. Helper phage M13KO7 is added to a final concentration of 2 x107 pfu/ml and the culture is incubated for 1.5 hours at 37°C with sufficient agitation (200-300 rpm) for good aeration of the culture. Kanamycin (25 mg/ml in water) is then added to a final concentration of 70 µg/ml. Incubation of the culture is continued overnight (12 to 15 hours) at 37°C.

2. Recover entire culture volume to a centrifuge tube or bottle and pellet bacteria by centrifugation at 4,000 x g (r_{max}) for 20 minutes, 4°C. Recover resultant supernatant to a fresh tube or bottle and re-centrifuge as above.
3. Recover resultant supernatant to a fresh centrifuge tube, add 0.25 volume of 20% PEG 8000/2.5 M NaCl to the supernatant, mix thoroughly by repeated inversion, and incubate 20 minutes at room temperature. Centrifuge at 15,500 x g, 4°C, for 20 minutes and discard the resultant supernatant. Allow any traces of supernatant to drain from the tube.
4. Resuspend pellet in 50 µl TE (10 mM Tris-Cl, 1 mM EDTA, pH 8.0) for every 1 ml of supernatant (step 3) and transfer the entire sample to a pre-spun (1500 x g for 1 to 2 minutes) PLG 15 ml Light tube.
5. Add an equal volume of Tris-buffer saturated Phenol (pH 8.0) to the sample in the PLG 15 ml tube, and mix thoroughly by repeated inversion. Centrifuge at 1500 x g for 5 minutes to separate the phases.
6. Add an equal volume of Phenol:Chloroform:Isoamyl Alcohol (PCI, 25:24:1) to the sample in the same PLG 15 ml tube and extract and centrifuge as in step 5.
7. Add an equal volume of Chloroform:Isoamyl Alcohol (CI, 24:1) to the sample in the same PLG 15 ml tube and extract and centrifuge as in step 5.
8. Quantitatively transfer final aqueous phase to a 2 ml microcentrifuge tube, add 3 volumes of a 25:1 mixture of absolute Ethanol: 3 M Sodium Acetate (pH 5.2), and mix well.
9. Let stand for 15 to 30 minutes at room temperature, and centrifuge for 20 minutes at 12,000 x g or greater at 4°C.
10. Wash pellet twice with 0.5 ml 70% Ethanol and air-dry.
11. Resuspend pellet in 20 to 50 µl TE, pH 8.0.

Lambda DNA extraction protocol

This method produces good quality λDNA with yields of 15 to 20 µg λgt10 DNA per 450 µl of supernatant processed. PLG tubes also can be used in conjunction with other λ DNA purification methods.[4]

1. Grow a 10 ml stock of suitable plating bacteria in L-broth plus Mg^{2+} (1% Tryptone, 0.5% yeast extract, 0.5% NaCl, 1 mM $MgSO_4$, pH 7.5) overnight at 37°C.
2. Dilute λ phage stock to 10^5 pfu per 0.1 ml in SM buffer (0.1 M NaCl, 8 mM $MgSO_4$, 50 mM Tris-Cl, pH 7.5, 0.01% Gelatin), then mix 0.1 ml of the λ dilution with 0.1 ml plating bacteria in a sterile 13 x 100 mm culture tube and allow phage to adsorb to bacteria for 20 minutes at 37°C.
3. Add 2.5 ml melted top agarose (L-broth plus Mg^{2+} and 0.6% agarose, 45 to 50°C) to the tube, mix well and pour onto a 100 mm L-broth plus Mg^{2+} plate. Allow top agarose to harden and incubate at 37°C for 8 hours. Plaques should be evident at this time.
4. Chill plate for 15 minutes at 4°C, add 3 ml λ diluent (10 mM Tris-Cl, pH 7.5, 8 mM $MgSO_4$) to the plate and incubate overnight at 4°C with gentle rocking.
5. Collect phage-containing λ diluent to a centrifuge tube and centrifuge at 4,000 x g for 10 minutes, 4°C, to pellet debris.
6. Transfer 450 µl of the resultant supernatant to a pre-spun (1500 x g for 1 to 2 minutes) PLG 15 ml tube (Light or Heavy), add 4.5 µl 1 mg/ml DNase λ and 2.0 µl 12.5 mg/ml RNase A, mix and incubate 30 minutes at 37°C.
7. Add 11.5 µl 20% SDS and 4.5 µl 10 mg/ml Proteinase K to the sample, mix, and incubate 30 minutes at 37°C.
8. Extract sample with 500 µl Phenol:Chloroform:Isoamyl Alcohol (PCI, 25:24:1) and centrifuge for 5 minutes at 1500 x g to separate the phases.
9. Repeat step 8 in the same PLG 15 ml tube.
10. Using the same PLG 15 ml tube again, extract once with 500 µl Chloroform:Isoamyl Alcohol (CI, 24:1) and centrifuge for 5 minutes at 1500 x g to separate the phases.
11. Transfer final aqueous phase to a 1.5 ml microcentrifuge tube, add 45 µl 3 M Sodium Acetate, pH 5.2 and 500 µl 100% Isopropanol, mix and incubate at room temperature for 15 minutes.
12. Centrifuge 20 minutes at 12,000 x g or more, discard the supernatant and wash pellet two times with 70% Ethanol prior to drying.
13. Resuspend the DNA in 20 to 50 µl TE (10 mM Tris-Cl, 1 mM EDTA, pH 8.0).

Genomic DNA isolation protocol[11,12,14]

→ Genomic DNA is fragile. High molecular weight DNA is sheared easily by mechanical forces. Use suitable large-bore pipette tips or equipment when pipetting genomic DNA. Do not vortex solutions containing genomic DNA.

Isolation of nuclei

Nuclei from blood

→ Caution: Wear suitable protective apparel and take appropriate safety measures when working with human blood.

1. Transfer 5 ml whole blood (containing EDTA as the anti-coagulant) to a 15 ml polypropylene centrifuge tube.
   → Note: When working with avian blood, or blood from other species that have nucleated red blood cells, use 0.2 ml blood and 4.8 ml 1x Tris-Buffered Saline (TBS, 50 mM Tris-Cl, 200 mM NaCl, 3 mM KCl, 0.02% Sodium Azide, pH 7.5) instead of 5 ml blood.
2. Add 5 ml 2x Lysis Buffer (0.65 M Sucrose, 20 mM Tris-Cl, pH 7.8, 10 mM $MgCl_2$, 2% Triton X-100) and mix by gentle inversion.
3. Incubate solution for 5 minutes on ice.
4. Pellet nuclei by centrifugation at 1000 x g for 12 minutes at 4°C.
5. Decant supernatant and drain any residual supernatant by inverting the tube on a paper towel for 2 minutes.
   → Note: The pelleted nuclei may be stored at -70°C for several weeks at this point, however, higher molecular weight DNA is obtained when genomic DNA is isolated from freshly prepared nuclei.

Proceed to lysis of nuclei and deproteinization of DNA.

Nuclei from tissue culture cells

1. Suspend cells from one 100 mm plate in the growth medium and transfer them to a 15 ml polypropylene centrifuge tube.
2. Pellet cells by centrifugation at 250 x g for 5 minutes at 4°C.
3. Decant supernatant and drain any residual supernatant by inverting the tube on a paper towel for 2 minutes.
4. Suspend cells in 1 ml 1x TBS by repeated up and down pipetting, then add 4 ml 1x TBS and 5 ml 2x Lysis Buffer to the cell suspension and mix by inversion.
5. After a 5 minute incubation on ice, pellet nuclei by centrifugation at 1100 x g for 12 minutes at 4°C.
6. Decant supernatant and drain any residual supernatant by inverting the tube on a paper towel for 2 minutes.

→ Note: The nuclei may be stored at -70°C for several weeks at this point, however, higher molecular weight DNA is obtained from freshly prepared nuclei.

Proceed to lysis of nuclei and deproteinization of DNA.

Lysis of nuclei and deproteinization of DNA

1. Suspend pelleted nuclei in 2 ml Saline/EDTA Solution (75 mM NaCl, 24 mM EDTA) by repeated up and down pipetting.
2. Carefully transfer suspended nuclei to a pre-spun (1500 x g for 2 to 3 minutes) PLG 15 ml Light tube.
3. Add 50 µl 20 mg/ml Proteinase K (in 10 mM Tris-Cl, pH 8.0, 1.0 mM $CaCl_2$, 30% Glycerol) and 200 µl 10% SDS to the suspended nuclei and mix by gentle inversion.
4. Incubate for 2 hours at 37°C with occasional gentle mixing.
   → Note: This incubation may be extended to 16 to 18 hours if that is more convenient. Proceed to extraction of protein.

Extraction of protein

1. Add 4 ml water-saturated Phenol to the PLG 15 ml Light tube containing the deproteinized DNA. Cap the tube tightly.
2. Mix by shaking vigorously enough to form a homogeneous suspension. Do not vortex.
3. Centrifuge at 1500 x g for 5 minutes to isolate the upper, DNA-containing aqueous phase away from the lower, organic solvent phase.
4. Carefully decant upper phase containing the DNA into a fresh, pre-spun, PLG 15 ml Light tube.
5. Repeat steps 1 through 3 but this time extracting with 4 ml water-saturated Phenol:Chloroform (PC, 1:1).
6. Carefully decant upper phase containing the DNA into a clean 15 ml polypropylene centrifuge tube.

Proceed to precipitation of DNA.

Precipitation of DNA

→ At this point, the DNA should have been extracted with both Phenol and Phenol:Chloroform and should be in a clean 15 ml polypropylene screw cap centrifuge tube.

1. Add 100 µl 2 M KCl and mix by gentle inversion.
2. Overlay DNA solution with 5 ml 95% Ethanol by slowly pipetting the Ethanol down the side of the tube.
3. Place a Pasteur pipette tip at the interface of the DNA-Ethanol solution and spool the DNA onto the pipette tip by swirling the pipette, keeping the tip at the interface, until the 2 phases are completely mixed.
4. Place pipette tip with the spooled DNA in 1 ml 70% Ethanol for about 2 minutes.
5. Remove pipette from the 70% Ethanol and hold upright (tip up) for a few seconds to allow the excess Ethanol to drain away. Do not allow the DNA to dry.
6. Set pipette tip in a microcentrifuge tube containing 200 µl TE (10 mM Tris-Cl, 1 mM EDTA, pH 8.0) and incubate for 20 minutes at room temperature. The DNA should slide from the pipette into the TE. If necessary, gently remove the DNA from the pipette by scraping the DNA onto the interior of the tube.
7. Redissolve DNA completely by incubating overnight at 4°C. Store DNA at 4°C. Do not freeze.

Mouse tail genomic DNA isolation protocol[13]

→ Note: Genomic DNA is fragile. High molecular weight DNA is sheared easily by mechanical forces. Use suitable large-bore pipette tips or equipment when pipetting genomic DNA. Do not vortex solutions containing genomic DNA.

1. Place a 1 cm tail sample into a 1.5 ml microcentrifuge tube; this may be stored at -20°C. To minimize possible cross-contamination, do not mince the sample. Add 700 µl Lysis Buffer (50 mM Tris-HCl, pH 8.0, 100 mM EDTA, 100 mM NaCl, 1% SDS) to the sample.
2. Add 35 µl 10 mg/ml Proteinase K to the sample and mix briefly.
3. Incubate at 55 to 60°C overnight with mixing. This step should result in the complete solubilization of the tail fragment. In the case of incomplete digestion, more Proteinase K can be added and the samples incubated for several more hours.
4. Add 20 µl 10 mg/ml RNase A (DNase-free) to the sample. Mix briefly and incubate at 37°C for 1 to 2 hours.
5. Transfer entire solution to a pre-spun (1,500 x g for 1 to 2 minutes) PLG 2 ml Heavy tube.

6. Add 0.5 ml Phenol:Chloroform:Isoamyl Alcohol (PCI, 25:24:1) to the sample in the PLG 2 ml tube and mix well by repeated inversion. Do not vortex.
7. Centrifuge at full speed (12,000 x g or greater) for 5 minutes in a microcentrifuge, then carefully transfer the resultant aqueous phase to a fresh pre-spun PLG 2 ml Heavy tube.
8. Add 0.5 ml Chloroform:Isoamyl Alcohol (CI, 24:1) to the sample in the PLG 2 ml tube and mix well by repeated inversion. Do not vortex.
9. Centrifuge at full speed (12,000 x g or greater) for 5 minutes in a microcentrifuge, then carefully transfer resultant aqueous phase to a fresh microcentrifuge tube.
10. Fill sample-containing tube with 100% Isopropanol and mix thoroughly by repeated inversion. Do not vortex. A visible DNA precipitate should form. Proceed immediately to step 11.
11. Recover DNA precipitate by touching it to a heat-sealed glass micropipette tip or by lifting the DNA with a yellow pipette tip and partial suction from a pipettor. Transfer the DNA to a 1.5 ml microcentrifuge tube containing 70% Ethanol. If DNA is not stringy, pellet by a brief, low speed centrifugation.
12. Wash DNA with the 70% Ethanol, then wash twice with 95% Ethanol.
13. Allow DNA to partially dry and then either transfer the DNA to a microcentrifuge tube containing 400 µl TE (10 mM Tris-Cl, 1 mM EDTA, pH 8.0) or add 400 µl TE to the DNA in the tube. Do not vortex or re-pipet to resuspend DNA.
14. Re-solubilize the DNA overnight (e.g. by rotation at 30-60 rpm). Re-solubilization may be facilitated by heating the sample at 50°C.

Basic plasmid DNA isolation protocol

→ PLG can be used in small and large scale protocols based on alkaline lysis procedure.[2,7,14] The basic protocol given here works with *E. coli* cultures grown for 12 to 14 hours in up to 500 ml of amplified or non-amplified LB or DYT, or in 250 ml Terrific Broth.

1. Pellet bacteria from the culture at 10,000 x g for 5 minutes at room temperature.
2. Resuspend bacterial pellet in a total of 15 ml 25 mM Tris-Cl/10 mM EDTA (pH 8.0). Pipet up and down or vortex as necessary to fully resuspend the bacteria.
3. Add 15 ml room temperature 0.2 N NaOH/1.0% SDS to the suspension. Mix thoroughly by repeated gentle inversion. Do not vortex.
4. Add 15 ml ice-cold 7.5 M Ammonium Acetate to the lysate. Mix thoroughly by repeated gentle inversion. Do not vortex.
5. Centrifuge at 15,500 x g for 30 minutes at 4°C.
6. Recover resulting supernatant and divide it between 2 pre-spun (1500 x g for 2 to 3 minutes) PLG 50 ml Heavy tubes.

7. Add 20 ml Phenol:Chloroform:Isoamyl Alcohol (PCI, 25:24:1) to the sample in each PLG 50 ml Heavy tube and mix thoroughly by repeated inversion. Do not vortex. Centrifuge at 1500 x g for 5 minutes to separate the phases.
8. Recover resultant aqueous phases to fresh pre-spun PLG 50 ml Heavy tubes and repeat step 7 above.
9. Recover resultant aqueous phases to fresh pre-spun PLG 50 ml Heavy tubes. Add 20 ml Chloroform:Isoamyl Alcohol (CI, 24:1) and extract as in step 7 above.
10. Recover resultant aqueous phases to suitable tubes and process or precipitate the samples as per your protocols and procedures.

RNA-free plasmid DNA isolation protocol

→ The following protocol is for use with 1 liter cultures grown in LB medium for 12 to 14 hours. It is also used for cultures grown for no longer than 12 to 14 hours in 0.5 liter of enriched growth media such as Terrific Broth (TB). The reagents used in the cleared lysate preparation (step 1-5) should be scaled down proportionately for smaller culture volumes as low as 200 ml.

1. Pellet bacteria from the culture at 10,000 x g for 5 minutes at 4°C.
2. Resuspend bacterial pellet in a total of 30 ml 25 mM Tris-Cl/10 mM EDTA (pH 8.0). Pipet up and down or vortex as necessary to fully resuspend the bacteria.
3. Add 30 ml room temperature 0.2 N NaOH/1.0% SDS to the suspension. Mix thoroughly by repeated gentle inversion. Do not vortex.
4. Add 30 ml ice-cold 7.5 M Ammonium Acetate to the lysate. Mix thoroughly by repeated gentle inversion. Do not vortex.
5. Centrifuge at 15,500 x g for 30 minutes at 4°C.
6. Recover resultant supernatant to a fresh centrifuge bottle. Do not carry over any whitish-grey pellet material.
7. Add 54 ml (0.6 x volume) room temperature 100% Isopropanol to the supernatant. Mix thoroughly by repeated inversion. Do not vortex.
8. Centrifuge at 15,500 x g for 30 minutes at 20°C. Discard resultant supernatant.
9. Add 25 ml 70% Ethanol to the pellet. Mix by repeated inversion and centrifuge at 15,500 x g for 5 minutes to re-pellet DNA.
10. Discard resultant supernatant, carefully aspirate any excess Ethanol, and dry the pellet for 15 to 30 minutes.
11. Add 1.5 ml TE, (10 mM Tris-Cl, 1 mM EDTA, pH 8.0) containing 50 µg/ml RNase A and 2.5 µg/ml RNase T1 to the pellet and mix gently to dissolve pellet.
12. Centrifuge for 1 to 2 minutes at 10,000 x g to consolidate sample, then transfer sample to a pre-spun (1500 x g for 1 to 2 minutes) PLG 15 ml Light tube.

→ Note: 5 to 10 µl of this suspension can be analyzed by 1% agarose gel electrophoresis to confirm that plasmid DNA is present.

13. Incubate for 15 minutes at 37°C in a water bath.
14. Extract sample once with 2.0 ml PCI (Phenol:Chloroform:Isoamyl Alcohol 25:24:1). Thoroughly mix the aqueous and organic phases by repeated inversion. Do not vortex. Centrifuge at 1500 x g for 5 minutes to separate the phases.
15. Add 2.0 ml PCI to the aqueous sample in the same PLG 15 ml tube and extract as in step 14.
16. Add 2.0 ml Chloroform:Isoamyl Alcohol (24:1) to the aqueous sample in the same PLG 15 ml tube and extract as in step 14.
17. Carefully transfer resultant aqueous phase (1.4 to 1.5 ml) to a suitable fresh tube and process or precipitate the sample as per your protocols and procedures.

Total RNA isolation protocol[5,6,8]

1. Sample preparation:
   → To extract RNA from washed and pelleted cultured cells, add 200 µl 4 M Guanidinium Isothiocyanate Solution (4 M Guanidinium Isothiocyanate, 25 mM Sodium Citrate, pH 7.0, 0.1 M ß-Mercaptoethanol) to 0.5 x 10^4 cells - 1 x 10^6 cells.

   → To directly extract RNA from cultured cells growing in monolayer, add 200 µl 4 M Guanidinium Isothiocyanate Solution directly to each well of a 6, 12 or 24 well plate. Add 100 µl of the 4 M Guanidinium Isothiocyanate Solution directly to each well of a 48 or 96 well plate.
2. Homogenize monolayer cells by pipetting the mixture up and down several times, taking care to "wash" cell material free from the culture dish, tube or well in the process. Homogenize washed, pelleted cells by pipetting the mixture up and down until the pellet is fully suspended. Use a small bore pipette tip to collect the cell homogenate.
3. Transfer all of the homogenate to a pre-spun (12,000 - 16,000 x g for 1 to 2 minutes) PLG 2 ml Heavy tube.
4. Add 20 µl (10 µl per sample for 48 or 96 well plates) 2.0 M Sodium Acetate, pH 4.0 to the sample, cap the PLG tube and mix briefly.
5. Add 200 µl (100 µl per sample for 48 or 96 well plates) water-saturated Phenol to the sample, cap the PLG tube, and mix thoroughly by repeated inversion. Do not vortex.
6. Add 60 µl (30 µl per sample for 48 or 96 well plates) Chloroform:Isoamyl Alcohol (CI, 49:1) to the sample in the same PLG tube and mix thoroughly by repeated gentle inversion. Do not vortex.
7. Incubate on ice for 10 minutes.
8. Centrifuge at 12,000 - 16,000 x g for 5 minutes in a microcentrifuge to separate the phases.

9. Add 200 µl (100 µl per sample from 48 or 96 well plates) Phenol:Chloroform:Isoamyl Alcohol (PCI, 50:49:1) to the aqueous phase in the same PLG tube. Mix thoroughly by repeated gentle inversion. Do not vortex.
10. Centrifuge at 12,000 - 16,000 x g for 5 minutes to separate the phases.
11. Collect resultant aqueous phase to an RNase-free microcentrifuge tube, add an equal volume of 100% Isopropanol, and mix by repeated inversion.
12. Centrifuge at 12,000 - 16,000 x g for 20 minutes.
13. Discard resultant supernatant and wash pellet several times with 200 µl 70% Ethanol, centrifuging 2 to 3 minutes at 12,000 - 16,000 x g to re-pellet the sample.
    - Note: Samples may be stored in the 70% Ethanol wash at this stage at -70°C or colder for extended periods.
14. Discard final wash and dry pellet at room temperature.
15. Re-dissolve pellet in a suitable volume (5 to 10 µl) of RNase-free water. Store the RNA solution at -70°C.
    - Note: Absorbance determinations should be performed in RNase-free TE (10 mM Tris-Cl, 1 mM EDTA, pH 8.0).15

Troubleshooting

Problem	Possible Cause	Resolution
PLG is not phasing properly	The formulation may not be correct.	Check the compatibility chart in section 7 for the correct formulation of PLG.
	The centrifuge speed may be incorrect.	Check the protocol to assure the centrifuge speed is correct.
	The PLG may have been frozen.	Store the PLG at room temperature.
	The PLG was not spun down to the bottom of the tube prior to the extraction.	Spin the PLG to the bottom of the tube before performing the extraction.
The PLG migrates to the top of both the aqueous and organic phases.	The formulation may not be correct.	Check the compatibility chart in section 7 for the correct formulation of PLG.
	The aqueous layer is denser than the PLG.	Remove the liquid under the PLG by piercing the PLG with a pipette tip. Use a second pipette tip to recover the liquid and transfer to another pre-spun PLG tube. Add Molecular Biology Grade Water or an appropriate buffer to dilute the sample. Continue as described in the protocol.
PLG remains on the bottom of the tube.	The formulation may not be correct.	Check the compatibility chart in section 7 for the correct formulation of PLG.
	The organic phase is not dense enough to remain below the PLG.	Add chloroform to increase the density of the organic phase.

Problem	Possible Cause	Resolution
PLG is phasing but does not appear uniform.		If the barrier is intact, proceed with the protocol. If there appears to be a hole or space in the barrier, retrieve the sample and place in a new PLG tube. Add chloroform and proceed according to the protocol.

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*Phase Lock Gel and its uses are covered under U.S. Patent Numbers 5,106,966 and 5,175,271.

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RNA-Isol Lysis Reagent	200 ml	2302700
Water, Mol Bio grade	1 l	2500000
Water, Mol Bio grade	10 x 50 ml	2500010
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